SCHOOL INTEGRATED PEST MANAGEMENT WITH EMPHASIS ON BIOLOGY AND CONTROL OF THE HUMAN HEAD LOUSE, *Pediculus capitis* De Geer

BY

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Abstract of Dissertation Presented to the Graduate School of the
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SCHOOL INTEGRATED PEST MANAGEMENT WITH EMPHASIS ON THE BIOLOGY AND CONTROL OF THE HUMAN HEAD LOUSE, *Pediculus capitis* De Geer

By

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Through a process of educational workshops, development of the National School Integrated Pest Management (IPM) WWW Site (http://www.ifas.ufl.edu/~schoolipm/), and production and distribution of training materials, the entire K-12 public school system of the state of Florida was targeted for IPM adoption. Surveys indicated the percentage of Florida school districts practicing IPM increased from just 46% in 1996 to 74% in 1999. Surveys also indicated a significant change in types of pesticides being applied and in the techniques used to apply pesticides. Cockroaches were reported as the top pest in 1996 while ants became the top pest in schools in 1999. Adoption of IPM seemed to have a slight positive correlation with schools contracting pest management services to private firms. Creation of a risk assessment model using spatial statistics allowed visualization of various risk and benefit factors with regards to pest management

in schools. The model validated the advantage of using two key concepts of IPM; monitoring and precision targeting.

A clinical trial was initiated to determine the treatment success of two of the most popular brands of head lice treatments. Approximately 70% of samples obtained from NIX®-treated children and 52% of samples obtained from RID®-treated children still contained live lice 24 hrs after treatment and represent treatment failure. The synergized pyrethrin in RID® did not improve performance over the non-synergized permethrin in the NIX® product, suggesting target site insensitivity or the *kd-r* mechanism of insecticide resistance.

Experiments examining the off-host survival of head lice indicated that adult lice died relatively quickly at 30°C (LT_{50} = 14 hrs) but survived as long as 48 hrs at room temperature (20 - 25°C). After being removed from the host, eggs of head lice do not hatch at 20°C but will hatch up to 9 days at 25 or 30°C.

Analysis of cuticular hydrocarbons using gas chromatography revealed unique hydrocarbon patterns for each of the two species of *Pediculus*. These results support the elevation of the human head louse and the human body louse to species level. In addition, unique morphological features of the head louse were documented through scanning electron microscopy and compound microscopy.

CHAPTER 1
LITERATURE REVIEW

Introduction

Humans have been afflicted with lice at least since the beginning of recorded history. The earliest recordings of man's history include descriptions of the parasitic insects. Lice are mentioned in the Bible and have been found on remains of Egyptian mummies (Ewing 1924), pre-Columbian inhabitants of Peru (Ewing 1924), early inhabitants of North America (Gill and Owsley 1985) and early settlers of 15th century Greenland (Bresciani et al.. 1983). The oldest known specimens of lice were removed from the remains of an individual who lived in an area near the Dead Sea 9,000 years ago (Zias and Mumcuoglu 1991).

As long as humans have been fighting lice they have made attempts at curing the infestation. Combs used to remove lice and lice eggs have been found in archeological digs and dated as far back as the 1st century BCE (Mumcuoglu and Zias 1988). Such combs are known to have existed for at least 3500 years.

Only until recent history have humans discovered that lice are not generated from dirt, disease, or decomposing sweat (Burgess 1995). This belief was widespread and included cultures from both the Orient and Europe (Hoeppli 1959). It was only after the invention of the microscope that man began to understand the biology of lice.

Biology of Lice

Taxonomy. Human lice are insects in the Order Anoplura. There are three types of lice known to infest humans (Burgess 1995). The crab louse or pubic louse, *Pthirus pubis* (Linnaeus), is in the family Pthiridae along with another species, *Pthirus gorrillae* Ewing, which infests apes. The two forms of lice known as body lice, *Pediculus humanus* L., and head lice, *Pediculus capitis* De Geer, are in the family Pediculidae. However, the accepted classification of these two lice species has a long history.

In 1758 Linnaeus named the human louse *Pediculus humanus,* providing no distinction between those populations of lice which inhabit the heads or those which inhabit the body and/or clothes of the host (Linnaeus 1758). De Geer (1778) described the head louse as *Pediculus humanus capitis* and the body louse as *Pediculus humanus corporis.* At least a dozen trinomials have been used to describe various populations of head or body lice based on difference in host race or pigmentation of the specimen (Farrenholz 1917). After a thorough review, Ferris (1935) combined these into one species.

Although body lice are generally considered to be slightly larger, morphological characters are of little value when comparing head lice and body lice because many morphological characters shared by both, overlap in size range (Buxton 1939, Ferris 1951). Bacot (1917) conducted many studies on the biology of the two forms of the species and found slight differences in survival, oviposition rates, and morphological features. Bacot also found that cross-mated head and body lice could produce viable offspring, suggesting that the two forms are the same species. Keilin and Nuttall (1919)

later pointed out that many of the hybrid specimens which Bacot produced were in fact hermaphroditic. Busvine (1948) reviewed all the available evidence at the time and believed that there was not sufficient evidence to promote the two types to separate species status, and concluded that they were subspecies. After another review of the evidence, Ferris (1951) stated that a single species name, *Pediculus humanus* Linnaeus, was sufficient and that use of the common names "head louse" and "body louse" was up to the researcher when he/she thought it necessary.

The taxonomic status of *Pediculus humanus* was relatively stable until Busvine (1978) provided new evidence to separate the two forms. Busvine found that the two forms retained their characteristic size differences even though they inhabited the same host. Examination of insects from the exact same host resulted in no intermediate sized individuals being discovered. This new evidence along with the behavioral ecology exhibited by these two forms led Busvine to declare them to be two separate species, *Pediculus capitis* and *Pediculus humanus*. Schaefer (1978) analyzed the data from Busvine (1978) focusing on ecological factors. He concluded that the two species existed in two different environments which produced different selection pressures which maintained the different forms of lice.

Apparently, many researchers do not agree with Busvine (1978) and Schaefer (1978) as many continue to use the trinomial (Zeichner 1999, Meinking 1999, Mumcuoglu et al. 1990a, Downs et al. 1999c, Price and Benitez 1989, Pitman et al. 1987, Urcuyo and Zaias 1986, Gratz 1985, Alexander 1984) or variety (Taplin et al. 1982, Taplin et al. 1986, Pray 1999, Chosidow et al. 1994) classifications. More recently Chirov and Ozerova (1997) reviewed the situation including the data contained within

Busvine (1978) and Schaefer (1978) and again concluded that there was enough biological evidence to support the concept of two species. Interestingly, the editorial committee of the journal agreed with Chirov and Ozerova in elevating head lice and body lice to species level but disagreed with the authors on which scientific names were appropriate for each based on priority. For the purpose of this document the author has chosen to use *Pediculus capitis* De Geer for the human head louse and *Pediculus humanus* L. for the human body louse.

Life Cycle. Much of the information provided here is summarized from Nuttall (1917a) and Buxton (1939). The body louse and head louse have a very similar life cycle with the exception of the egg stage. The female head louse oviposits eggs on hairs of the scalp of its host. The female body louse oviposits eggs on the clothing of its host.

Specially adapted structures called gonopods on the ventral side of the terminal end of the abdomen align a single human hair to the ovipositor of the female. Almost immediately as the egg exits, a specialized gland located at the ovipositor secretes an adhesive which instantly glues the egg to the human hair (Nuttall 1917a, Schmidt 1939). In the body louse the process is identical except that the egg is deposited on fibers of clothing. The exact composition of the adhesive has remained a mystery (Schmidt 1939) until recently. The adhesive is reported to be comprised primarily of amino acids (Burkhart et al. 1998, Burkhart et al. 1999). Because no solvent for the glue exists, mechanical means for egg removal have been developed (Gerberg 1973).

Eggs of the head louse hatch within 7-10 days when exposed to the normal temperature of 30 °C. Body lice eggs require a slightly longer incubation period of 10-12 days. There are three nymphal stages in both species which last approximately 3-4

days each. Upon reaching the adult stage, females have the capacity to lay eggs within 1-2 days. Adults of both species live approximately 25-30 days. The total egg output has been estimated as 100-150 for the head louse and 250-300 for the body louse (Buxton 1939).

Many early researchers were interested in the effect of temperature on lice survival and egg hatch. Quick kill of eggs can be achieved by exposure to 55° C in 10 min. (Nuttall 1917a) while eggs exposed to -17° C will die after 2 hrs. (Chung 1937). Lang (1975) determined that the highest temperature under which head lice eggs will hatch is 39° C and the lowest temperature 24° C.

Adult body lice can survive only 2 days away from the host at 30° C, but as many as 7 days when held at 10° C. Head lice are more susceptible to off-host conditions (Busvine 1948, Bacot 1914). Busvine reported (1948) starved head lice survived between 24 and 40 hrs at 30° C and 75% RH; however, Lang (1975) reported survival to be only 24 hrs at a temperature of 26° C and 75% RH. Body lice adults will die in 1 hr when exposed to 47° C (Buxton 1939) and head lice die in 30 min exposed to the same conditions (Lang 1975).

Ecology. Lice in the Order Anoplura are obligate, permanent parasites and live exclusively on mammals. There are almost 500 described species of Anoplura resulting in a world-wide distribution (Kim et al. 1986). It is believed that the Anoplura evolved from early forms of Psocodea during the Jurassic period when the first mammals appeared (Kim 1986). These early Anopluran species adapted to the skin-fur environment of their hosts and fed primarily on dead skin and the like. As these early mammals evolved so did their parasites. By the Paleocene and Eocene periods the

obligate parasites had evolved to feed on the blood of their hosts (Kim and Ludwig 1978).

It is generally considered that as humans began losing body hair and wearing clothing the lice infesting them adapted. The addition of clothing did not affect the head louse but caused the body louse to adapt to a slight change in micro-climate and egg laying behavior (Nuttall 1919b). Therefore, the head louse is considered the more primitive of the two species (Nuttall 1919b, Zinsser 1945).

Human body lice inhabit the clothes of its host, only crawling to the skin of the host for blood meals. Body lice normally take 5-6 blood meals daily but can be reared in the laboratory when provided only 2 blood meals daily (Culpepper 1944). Using this method a laboratory colony of body lice was established removing the lice from rearing chambers and allowing them to feed on humans twice per day. Over time the lice were transferred to be reared solely on rabbits (Cole 1966). This is currently the only known method of rearing human lice in the laboratory.

Human head lice live their entire life cycle on the human host. Eggs are attached to hairs which upon hatching remain on the hairs. These "nits" remain until they are physically removed by the host (either with nit combs or by cutting the hair). Upon eclosion, the 1st stage nymph immediately begins searching for a blood meal. As with the body louse, the head louse requires several blood meals each day. Most of the time nymphs and adults of the head louse can be found crawling among hairs very near the scalp. Only when adults become old, exhausted, affected by insecticide, or otherwise exhibit abnormal behavior, will they be found on the outer surface of the host's hair for extended periods of time (Maunder 1988).

There are no known predators or parasites of head lice or body lice with the exception of a few microorganisms that can cause disease in humans. Buxton (1939) noted that in some parts of the world, infested persons found some relief by placing their clothes on ant nests and allowing ants to carry off the lice. It is known that adult lice eventually expire from "old age" after approximately 30 days. Buxton stated that the most influential factor controlling lice populations is the host. He suggested that the behavior of washing, combing, grooming, and laundering is the most critical factor contributing to lice mortality.

Epidemiology

Body lice have historically been associated with humans who practice poor personal hygiene (Burgess 1995), although they once were considered a sign of good health in New York (Sobel 1913), somehow beneficial to the Irish (Knott 1905), and may still provide perceived comfort to Papago and Yaqui Indians (Lang 1975). Lice were very common throughout the world until the 19th century when humans began to bathe and change clothes regularly (Zinsser 1945), and are now considered to be maintained only on homeless individuals, refugees, or other people living under very poor environmental conditions (Gratz 1997).

Body lice are known to vector three bacteria among humans: relapsing fever (pathogen = *Borrelia recurrentis*) (Mackie 1907), epidemic typhus (pathogen = *Rickettsia prowazeki*) (Nicolle et al. 1909, Ricketts and Wilder 1910), and trench fever (pathogen = *Bartonella quintana*) (Hurst 1916). The bacteria are transmitted when an

infected louse or its feces are crushed or rubbed into the bite site or other excoriations of the skin.

Epidemic typhus has played a major role in the history of civilization. Typhus has afflicted entire cities and even caused more fatalities than either army during Napoleon's war with Russia (Zinsser 1945). During the years of World War I it was estimated that epidemic typhus killed approximately 3 million Russians (Patterson 1993). Today, louse-borne diseases are restricted to those small communities around the world still struggling with poverty (Rydkina et al. 1999, Roux and Raoult 1999) or poor hygiene caused by civil unrest such as in Central Africa (Sundnes and Haimanot 1993).

Head lice are not known to vector any disease although theoretically it is possible (Maunder 1983). Head lice are mostly a problem for school-aged children (Downs et al. 1999a). One of the earliest reports on head lice infestations was conducted in 1898 (Greene 1898) when nearly 73% of children in a single Boston school were found to have at least a "few" nits in their hair. The first comprehensive study found that between 22-23% of all school children were infested with head lice consistently over a 4 year period (Sobel 1913). Many studies have been conducted since reporting various infestation levels from across the U.S.: Georgia 3% (Slonka et al. 1976), Florida 6.7% (Price and Benitez 1989) Florida, Georgia and New York 8% (Juranek 1977), Washington D.C. 20-25% (Murphy 1943), and around the world: Canada 2.5% (Ewasechko 1981), Australia 21% (Speare and Buettner 1999), Poland 0.1 - 4.65% (Lonc and Okulewicz 2000), England 14 - 52% (Mellanby 1942), Ghana 50% (Kwaku-Kpikpi 1982), Israel 15 - 20% (Mumcuoglu et al. 1990b) and Pakistan 46% (Suleman and Fatima 1988).

Factors such as race, gender, and hair length may influence prevalence of head lice only locally. Head lice become very closely adapted to their local hosts (Ferris 1935) such that claw size and shape will become adapted to a specific hair type (Ashcroft 1969, Maunder and Bain 1980) of the predominant host race. This phenomenon explains why in the United States very few black children become infested (Murphy 1943, Slonka et al. 1976, Price and Benitez 1989). However, in Black African populations head lice infestation rates mirror those in the U.S. (Iwuala and Onyeka 1977, Kwaku-Kpikpi 1982).

Most studies have indicated that females are infested at higher rates, and most researchers suggest that this is due to behavior (Burgess 1995). School-aged girls are engaged in more head to head contact and tend to play in close, small groups (Burgess 1995). Hair length has been both positively correlated (Suleman and Fatima 1988, Kwaku-Kpikpi 1982) and negatively correlated (Mumcuoglu et al. 1990, Maunder 1993) to head lice infestation rates.

General cleanliness has certainly resulted in fewer people being infested with body lice (Gratz 1997), but personal hygiene does not seem to be as important with head lice (Burgess 1995). Although some reports exist documenting higher infestation rates associated with lower socioeconomic status (Juranek 1977) the exact opposite has also been observed (Mumcuoglu et al. 1992). Chunge (1986) reported that the chances of becoming infested were equal for children with clean hair or dirty hair.

Treatment and Control

The earliest known control measures employed against human lice involved the use of combs or simply fingers to remove nits (Mumcuoglu 1996). Later attempts involved shaving the body and head (Buxton 1939) or use of botanicals (Hoeppli 1959). Some of the more popular botanicals included extracts from *Stemosa tuberosa* (Hoeppli 1959), a *Delphinium* species (Busvine 1946), sabadilla, tobacco (Lindsay 1993), and *Derris* and *Lonchocarpus* (Rotenone) (Murphy 1943). Various oils have been formulated over the years including sassafras (Scobbie 1945) and eucalyptus (Peters 1922). Oil of petrolatum and related agents, used as a component of hair styling in some cultures, have been suggested as having pediculicidal activities (Greene 1898) but others disagree (Burgess 1995). Even though effective chemical treatments have been developed, many communities still rely on mechanical removal because modern treatments are too expensive, not available, or parents have grown concerned about chemical exposure to their children (Burgess 1995).

Chemical treatments. Early chemical treatments include kerosene (Jamieson 1895, Greene 1898), xylene (Ferguson 1930), mercury compounds (Buxton 1939), and thiocyanate oils (Lethane®) (Busvine and Buxton 1942). Although these compounds provided some relief, they each came with serious side effects. The first major advancement in lice control, and for the control of many other injurious insects, was the development of DDT (Buxton 1945). DDT was used extensively during World War II as a dust formulation to prevent typhus (Wheeler 1946, Burgess 1995). Unfortunately resistant populations of body lice soon appeared (Hurlburt et al. 1952, Kitaoka 1952,

Wright and Brown 1957), and the need for additional treatments stimulated the development of many chemical treatments over the second half of the 20th century.

Lindane (Busvine 1946), malathion (Barnes et al. 1962), carbaryl (Clark and Cole 1967, Maunder 1981), pyrethrins, and pyrethroids (Taplin and Meinking 1990) have all been formulated and marketed as dusts, lotions or shampoos for treatment of human lice. Malathion (Petros 1990) and lindane (Ginsberg and Lowry 1983) have been associated with serious side effects. Therefore, pyrethrins or pyrethroid formulations are the current preferred products for parents of infested children (Maunder 1991) in developed countries.

Over the past 15 years pyrethroid-containing products have become the most preferred products among parents for treatment of pediculosis (Burgess 1995, Meinking 1999). Recent reports have indicated that wild head lice populations exhibit decreased levels of susceptibility to many pediculicides including pyrethroid insecticides (Chosidow et al. 1994, Downs et al. 1998, Pollack et al. 1999). Documented pyrethroid resistance in head lice (Mumcuoglu et al. 1995, Picollo et al. 2000) is possible cause for recent increases in head lice problems. The current situation has compelled some researchers to look towards the development of new compounds for use against head lice, such as fipronil and imidacloprid (Downs et al. 1999c, Downs et al. 2000a).

Integrated Pest Management (IPM)

History of IPM. During World War II synthetic pesticides such as DDT and chlorinated hydrocarbons were developed and provided excellent control of public health pests. During the 1950's and 1960's hundreds of new insecticides were developed to

improve agriculture around the world. Strict reliance on synthetic insecticides to control pest insects resulted in the development of resistance in agricultural pests (Brown 1971) as well as in public health pests (Hurlburt et al. 1952) within a few years after introduction of a new compound (Brown and Pal 1971). In addition to the problems of insecticide resistance, concerns surrounding the impact of synthetic pesticides on the environment (Carson 1962) compelled researchers to develop improved management techniques that incorporate all risks and benefits of pest management (Luckman and Metcalf 1994). Concurrently, economic injury levels were used in agronomic crops to help determine the precise timing and method of control to optimize benefit (Pedigo 1989). Ultimately the term Integrated Pest Management (IPM) evolved to describe the holistic concept of "use of multiple tactics in a compatible manner to maintain pest damage below the economic injury level while providing protection against hazards to humans, animals, plants, and the environment" (Luckman and Metcalf 1994). Such tactics integrated a variety of control methods including cultural, mechanical, physical, biological, chemical, genetic, and regulatory (Metcalf et al. 1951).

IPM in the Urban Environment. Owens (1986) first described IPM in the urban environment as a process consisting of five steps: monitoring of pests, identification of pests, determining if pest levels exceed limit, applying pest management procedure, and evaluation/follow-up. Monitoring of pests helps to determine what kind of pest is present, where it is present, and the level of infestation. This information allows the pest manager to apply pesticides only when necessary (Robinson and Zungoli 1995). When application of pesticides is deemed necessary, an IPM program would utilize least hazardous, selective materials that still provide control of the pest (Olkowski

et al. 1991). Additionally, steps can be taken to reduce the potential hazard of human exposure to pesticides used indoors through precision targeting of pesticides (Brenner 1998) to surfaces not accessible to humans. Long-term pest management is often associated with improved sanitation and other preventative measures (Marsh and Bertholf 1986).

IPM in Schools. In 1993 the National Academy of Sciences published a report indicating that children were at a higher risk to pesticide exposure than were adults due to their smaller body size, higher metabolism, developing immune systems, and playful behavior (NRC 1993). In response to this report and many other concerns, the U.S. Congress passed the Food Quality Protection Act (FPQA) in 1996. As mandated by FQPA, the U.S. E.P.A. began reviewing the registration of many pesticides in hopes of ensuring the safety of compounds used for pest control in agriculture as well as in homes and schools. Currently, there are over 3000 labels for pesticides used in the school environment. A recent report by the U.S. General Accounting Office (GAO 1999) indicated a complete lack of information exists regarding what kind of pesticides are used in schools, how much of these pesticides are applied in schools, and where specifically they are being applied in schools. Because children between the ages of 5 and 18 spend approximately 1/6 of their lives in schools, many schools have restricted the use of pesticides on school grounds (Owens and Feldman 1998), and beginning in the early to mid 1990's many school districts began considering implementing IPM within their schools (Owens and Feldman 2000).

The interest in encouraging schools to adopt IPM has increased considerably since the US EPA published the first *School IPM Handbook* (EPA 1993). With increased

interest in School IPM came the need for increased and targeted training materials. Training materials have now been developed for Illinois (Anonymous 1994), Texas (Merchant 1996), New York (Stauffer et al. 1998), Tennessee (Vail 1998), Maryland (Davidson et al. 1998), and Florida (Koehler et al. 1998), and many others are in progress. Professional entomologists have been engaged in the issue of IPM in schools, and many are currently involved in adoption and training procedures across the country (Green 2000).

Although the term IPM is old in traditional agricultural arenas, it is relatively new to most indoor applications. The concept of preventing pests and managing pests through a variety of means and using pesticides judiciously is almost completely new to most individuals conducting pest management in schools. However, the United States Federal Government may force the adoption of School IPM nation-wide in the near future (SEPA 1999).

CHAPTER 2
OBSERVATIONS ON THE MORPHOLOGY OF THE HUMAN HEAD LOUSE

Introduction

The human head louse, *Pediculus capitis* De Geer, is a major pest among young children worldwide (Gratz 1997). Much of the biology, life history, and morphology of the head louse was described by Nuttall (1917b, 1919a), Bacot (1917), and Buxton (1939). However, improvements in technology now allow the use of high magnification microscopy to create high quality images documenting additional morphological features. It is the purpose of this study to document important morphological features of the head louse and possibly to provide additional insight into understanding the biology of this ubiquitous insect.

Materials and Methods

Specimens. Adults and eggs of head lice were obtained from consenting children in Alachua County (Florida) schools during regular head lice screenings in conjunction with school nurses' activities. Children were inspected by parting folds of hair beginning with the back of the head and systematically rotating around to include the entire scalp. Adult head lice specimens discovered through this procedure were removed using feather-touch forceps and initially placed in scintillation vials along with a small bit of hair. Head lice eggs were removed from infested children using a specialized "nit" comb,

The LiceMeister™ (National Pediculosis Association, Boston, MA). The design of this device is such that it efficiently removes head lice eggs from the hair shaft, although sometimes a bit of the hair shaft will be removed along with the egg. Once removed from the head, eggs were placed into a plastic Petri dish (15 x 100 mm). On two occasions the child's entire head was shaved using a barber's electric hair clippers, and the resulting hair mass recovered and returned to the laboratory for separation of eggs and live head lice.

Preparation of Specimens for Scanning Electron Microscopy. Three methods of preserving head lice specimens were compared to determine the best method. The first method involved placing live or recently killed head lice adults and eggs in a 1% glutaraldehyde solution (McDowell and Trumps fixative) as a preservative until ready for final processing. For final processing, specimens were removed from the McDowell and Trumps solution and dehydrated through a series of increasing concentration of ethyl alcohol as follows: 50%, 60%, 70%, 80%, 90%, 95%, and finally 100%. Specimens were placed into a 25 ml scintillation vial containing one of the concentrations of alcohol. Specimens were placed into each concentration for approximately 10 min then moved to the next highest concentration using a pipette. Specimens submerged in 100% ethyl alcohol were then placed into a Bal-Tec (Leichtenstein) CPD-030 critical point dryer as a final dehydration step.

The second method involved placing live or recently killed specimens directly into 70% ethyl alcohol until ready for final processing. For final processing, specimens

were dehydrated through a series of ethyl alcohol concentrations starting with 80% as described above and ultimately dried in the critical point dryer.

The third method for preparing specimens was identical to the second method except that specimens were initially preserved in 50% ethyl alcohol. Specimens were then dehydrated through a series of alcohol solutions beginning with 60%.

Head lice specimens from all three preparation methods were placed on SEM mounting stubs using double sided tape. Specimens on stubs were then sputter coated with gold/palladium using an Anatech Hummer VII Sputter Coater (Springfield, VA).

All specimens were observed using a Hitachi S-4000 FE-SEM (Tokyo, Japan) using a range of magnification from 40 to 1000 times. Micrographs were recorded digitally as "tif" files directly from the microscope using a digital capture system from Advanced Database Systems (Los Angeles, CA).

Preparation of Specimens for Compound Microscopy. Live adults and nymphs of head lice were obtained during the above procedure and placed into 25 ml scintillation vials until dead from starvation. Additional nymphs were also obtained from eggs which hatched in the laboratory. Specimens were placed onto glass microscope slides (Fisherbrand) with cover glasses using Euparol (Bioquip, Gardena, CA) as a mounting medium. Upon placing specimens on glass slides, slides were placed onto a slide warmer at 40^0 C for 1 week. Slide specimens were observed using an Olympus BH-2 Epifluorescent microscope (Tokyo, Japan). Photographs were recorded digitally as "tif" files directly from the microscope using a Pixera 120C Digital Camera (Los Gatos, CA).

Preparation of Specimens for Dissecting Microscopy. Live head lice specimens obtained through the above procedure were reared temporarily in a plastic Petri dish (15 x 100 mm). A single piece of Whatman paper (75 mm diameter) placed on the bottom of the petri dish provided the head lice with a substrate to crawl on. Petri dishes containing live head lice were held at room temperature (about 24° C). Live head lice were removed from the petri dish and placed on back of the author's hand and allowed to feed approximately 2 to 3 times a day (approximately 15 min each feeding) for a maximum of 4 days. This method of temporarily rearing head lice was suitable for keeping head lice alive long enough for dissecting microscope photography. During examination and photography, live specimens were transferred from the Petri dish onto the author's hand and observed using a Leica Wild M8 (Solms, Germany) dissecting microscope connected to a Leica Wild MPS52 camera body with a Leica Wild MPS48 computerized capture device. Kodak Elite Chrome 160T Tungsten slide film was used.

Results and Discussion

The best method for preparing head lice specimens for electron microscopy was method three, the process beginning with 50% ethyl alcohol and ending with critical point drying before sputter coating. All micrographs which appear in this document were recorded from specimens preserved in this fashion. The other two methods as described earlier in this chapter resulted in specimens of inferior quality.

An adult male head louse appears in Fig. 2-1. The light yellowish-tan to translucent appearance of the insect and its relative small size (1.5 - 2.5 mm) make it very

difficult to see when in its natural environment of the human scalp. The color of the gut contents will influence the appearance of lice. The dark coloring along the edge of the thorax is highly variable. Nuttall (1919a) studied pigmentation in human lice extensively and determined that environmental factors such as the color of the background (or habitat) on which lice nymphs are reared influenced the degree of pigmentation. Lice nymphs which were reared on light-colored surfaces resulted in light-colored adults and vice versa. Figure 2-2 shows the contrasting colors of two 1st instar nymphs. The nymph in the foreground of the photograph has digested a previous meal and appears darker in color whereas the nymph in the rear of the photograph is taking its first blood meal and appears bright red. Many researchers have observed the phenomenon of starved lice consuming such a large blood meal that the gut ruptures. Once the gut ruptures, blood enters the hemocoel and moves into almost all parts of the body. Lice will live for only about a day after this event.

A facial view of a male adult head louse is represented in Fig. 2-3. Being dorso-ventrally flattened and having enlarged claws on each leg most certainly contributes to one of the common names given to human lice, "crabs." Also in plain view in Fig. 2-3 are the primary sensory organs of the head louse, the antenna. Although head lice have eyes, it is believed that they serve little function other than detecting light and dark. Chemoreceptors on the antenna have been suggested as playing a primary role in detecting humidity, locating preferred feeding sites, and detecting odors (Nuttall 1917b, Wigglesworth 1941, Peock and Maunder 1993). In Fig. 2-4 the tiny sensory organs (peg organs) located on the distal end of a head louse antenna are visible. The basal portion of

the peg is porous and also serves as a chemoreceptor (Slifer and Sekhon 1980). Taxonomists have used the morphology of the antenna to distinguish between head lice (*Pediculus capitis* De Geer) and body lice (*P. humanus* L.). Specifically the 3rd antennal segment of head lice (Fig. 2-5) has been reported to be shorter than that of the body louse (Fig. 2-6) (Ferris 1935, Busvine 1948, Chirov and Ozerova 1997).

Figure 2-7 shows the everted mouthparts of the head louse. The short haustellum terminates with several denticles which are used to help anchor the haustellum to the surface of the skin. Once anchored, the internal stylets (not shown) are everted from within the head through the haustellum, pierce the surface of the skin and allow uptake of the host's blood. The internal stylets are visible in Fig. 2-8 in the inverted position. Posterior to the stylets lies the anterior portion of the pharyngeal pump. This organ is responsible for generating a slight vacuum facilitating the uptake of blood (Peacock 1917).

Head lice, like most insects, have spiracles through which air enters the trachea for gas exchange. In Fig. 2-9 the "honeycomb" nature of the internal portion of the spiracle is visible. It has been reported that this physical structural pattern provides the most efficient mechanism for gas exchange (Meinking and Taplin 1995). Lice possess the ability to close the spiracles and survive submersion in water for several hours (Nuttall 1917b). The spiracle in Fig. 2-9 is that of a head louse nymph. The tissue inside the spiracle which appears lightened or highlighted is considerably thinner than that of the surrounding surfaces and became "burned" by the SEM under high magnification while capturing the micrograph.

Also located on the thorax are the claw-like legs of the head louse (Fig. 2-10). These claws are specially adapted for grasping hairs of the human head and so are not well adapted for locomotion on hard surfaces such as tables, desks, hard floors, or counter tops. It has been reported that the size and shape of the head louse claw depends on the local population of human hosts to which the lice population has adapted (Ferris 1935, Ashcroft 1969). As the size and shape of the hair varies, so does the size and shape of the claws of infesting head lice.

An adult female head louse appears in Fig. 2-11. Branches of the trachea can be seen along the lateral edges of the abdomen connecting to the outside at each spiracle location. Also apparent in this figure is the presence of a developing egg. Female head lice may have as many as three eggs developing within their abdomen at one time (Nuttall 1917b); however, one or two are more common. Eggs are always oviposited with the operculum-containing end exiting last such that oviposition results in the operculum pointing towards the distal end of the hair. The female head louse has specially adapted gonopods which aid in lining up the ovipositor with an individual hair shaft (Fig. 2-12). These structures are triangular in shape and are located on the ventral surface just slightly anterior to the vaginal orifice.

From a dorsal view, the pointed shape of the external genitalia of the male head louse is visible (Fig. 2-13). The male head louse uses the dilator structure to enter the female and prepare the vagina for penetration with the male's penis (Nuttall 1917b). In females the abdomen terminates with the v-shaped valves of the ovipositor (Fig. 2-14).

The typical condition present in chronically infested children is exhibited in Fig. 2-15. Hundreds and possibly thousands of viable and non-viable eggs appear within the hair of this child. Although eggs of head lice vary in color (Burgess 1995), the gray to black appearance of these eggs appear in contrast to the light blond color of the infested child's hair. Based on the location of these eggs on the hair shafts relative to the scalp, most of the eggs in this photograph are probably viable. Children infested with this many eggs (Fig. 2-15) are relatively uncommon. The typical light (and recent) infestation of head lice is usually associated with fewer eggs. Also common in chronically, heavily infested children is the condition where multiple eggs have been oviposited on the same individual hair shaft (Fig. 2-16). The egg lowest on the hair shaft was the most recently oviposited whereas the egg highest on the hair shaft is the oldest. At the base of the lowest egg, the glue which is used to fasten the egg to the hair shaft is visible. The glue surrounds the basal one third of the egg and provides for a cup-like fastener (Gerberg 1973).

From an epidemiological perspective it is important to identify children with active infestations. It is not uncommon for medical practitioners or parents to misdiagnose an active infestation of head lice (Pollack et al. 2000). One of the primary reasons for this is the inability to distinguish between viable eggs and non-viable eggs. Eggs may be termed non-viable if they have already hatched or the developing embryo did not complete development and died. Figure 2-17 shows a comparison of the various colors and appearances of viable eggs and non-viable eggs. It is difficult to see the difference with the naked eye, especially if the eggs remain on an infested child. A closer

look at an egg in early to mid stage development (1 - 3 days) appears in Fig. 2-18. An egg in late stage development (6 - 8 days) appears in Fig. 2-19 where many features of the young insect's body are discernable. Temperature is very important for the proper development of a head louse embryo (Buxton 1939). When exposed to unfavorable conditions the embryo does not develop completely and often cannot successfully escape the egg upon hatching as shown in Figs. 2-20 and 2-21. This condition is common when eggs are incorrectly reared artificially (Meinking 1999).

Head lice molt three times before maturing into adults. First, second, and third stage nymphs appear in Figs. 2-22, 2-23, and 2-24, respectively. Head lice nymphs are generally indistinguishable from body lice nymphs but are reported to be slightly shorter in overall length (Buxton 1938a, Lang 1975). A male and female head louse appear in Figs. 25 and 26 respectively. Female head lice tend to be slightly larger than males.

Although many of the morphological features described in this chapter have been discussed previously (Nuttall 1917b, Peacock 1918) newer technology has allowed improved images to be developed as shown in this chapter. The head louse continues to be a pest of humans and a better understanding of its morphology and the function of such structures should aid in developing improved control strategies.

Fig. 2-1. A live male adult head louse.

Fig. 2-2. Two 1st instar nymphs taking a blood meal. The nymph in the foreground has fed previously whereas the other nymph is feeding for the first time causing it to appear bright red.

Fig. 2-3. Scanning electron micrograph (frontal view) of an adult male head louse.

Fig. 2-4. Scanning electron micrograph of the distal end of an adult head louse antenna.

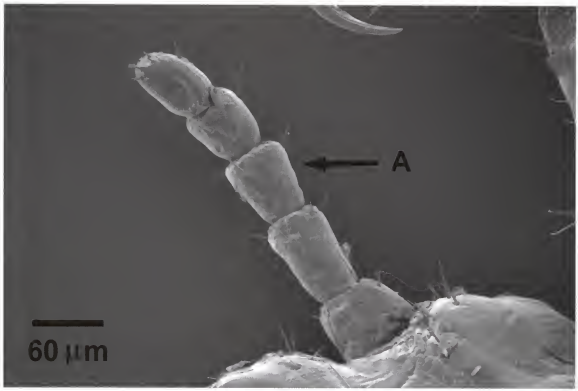

Fig. 2-5. Scanning electron micrograph of a head louse antenna showing relative size of the 3rd antennal segment (A).

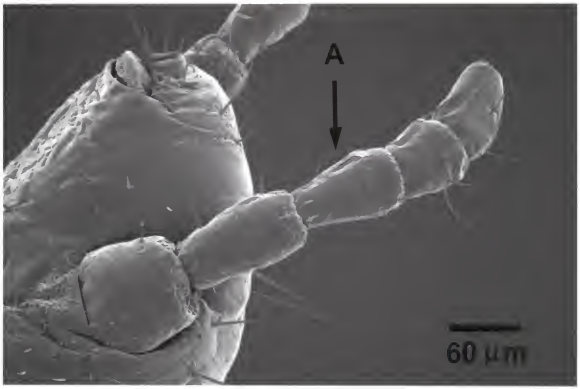

Fig. 2-6. Scanning electron micrograph of a body louse antenna showing the relative size of the 3rd antennal segment (A).

Fig. 2-7. Scanning electron micrograph of the everted haustellum of an adult head louse.

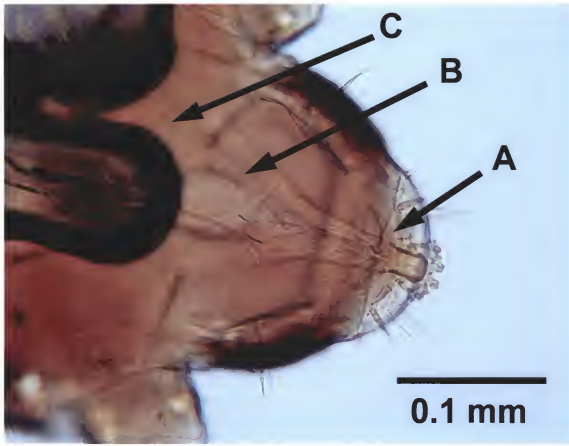

Fig. 2-8. The internal mouthparts of the head louse showing stylets (A), anterior portion of pharyngeal pump (B), and air bubble or artifact (C).

Fig. 2-9. Scanning electron micrograph of a thoracic spiracle of a head louse nymph.

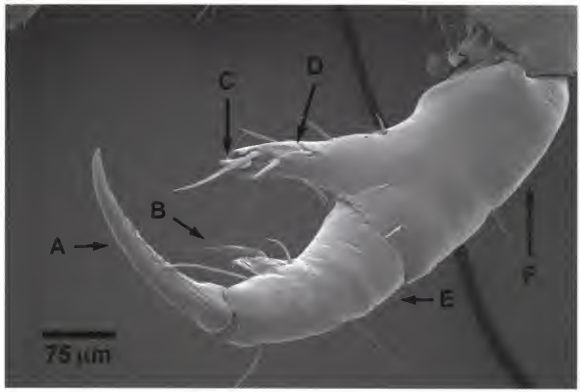

Fig. 2-10. Scanning electron micrograph of the front leg of a male head louse. The claw is specifically adapted for grasping hairs of the human head. Characters of the leg include the claw (A), lamella (B), spine of the thumb (C), "thumb" (D), tarsus (E), and tibia (F).

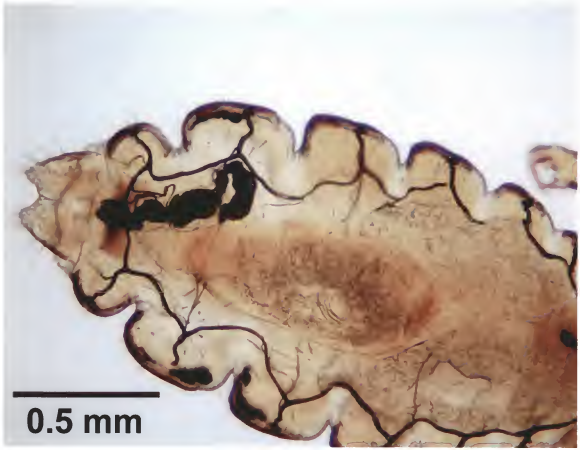

Fig. 2-11. Ventral view of the abdomen of a female head louse showing tracheal system and a developing egg.

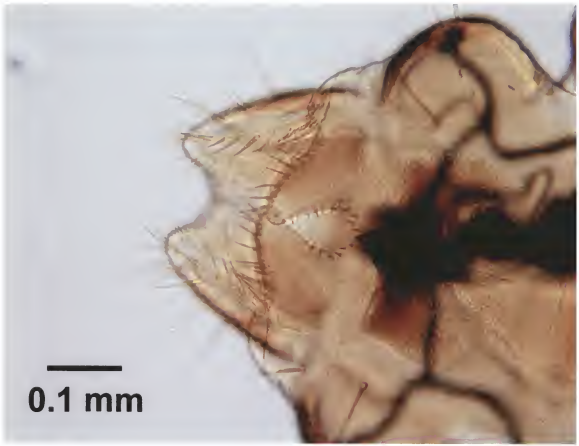

Fig. 2-12. Ventral view of the abdomen of a female head louse showing gonopods used to grasp a single hair shaft during oviposition.

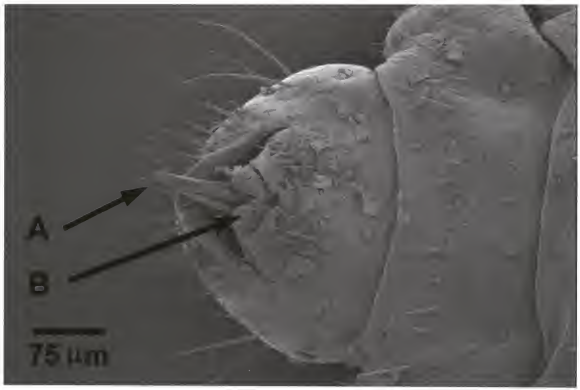

Fig. 2-13. Scanning electron micrograph (dorsal view) of the abdomen of an adult male head louse showing the dilator (A) and anal orifice (B).

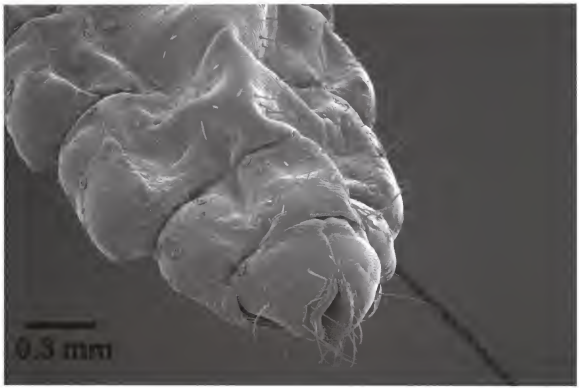

Fig. 2-14. Scanning electron micrograph (dorsal view) of the abdomen of an adult female head louse.

Fig. 2-15. Viable nits in the hair of a chronically infested child. The small dark spots in the child's hair are viable head lice eggs. In this case the child's light colored hair contrasts considerably with the color of the eggs.

Fig. 2-16. Scanning electron micrograph of a single hair strand with three eggs attached. The open operculum on each egg indicates it has hatched.

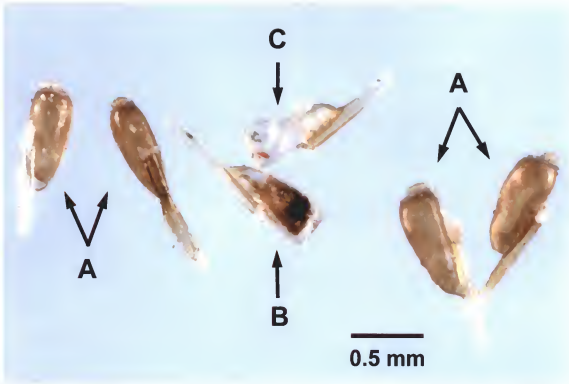

Fig. 2-17. Head lice eggs removed from an infested child's head. Tan to brown eggs (A) are viable eggs. Dark brown to black eggs (B) contain embryos which failed to properly develop. Clear or white eggs (C) are empty egg shells that previously hatched.

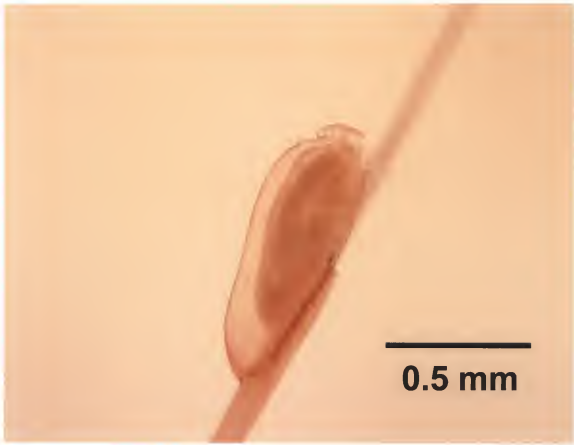

Fig. 2-18. A head louse egg in early to mid stage development. The dark mass within the egg is a developing embryo.

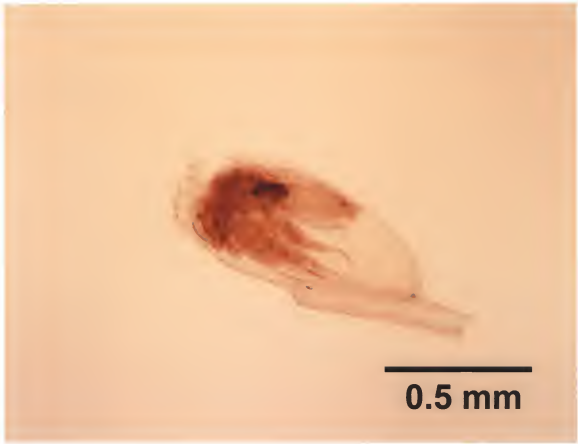

Fig. 2-19. A head louse egg in late stage development with legs, abdomen, head, and thorax region of the embryo visible through the egg shell.

Fig. 2-20. A head louse nymph that was unable to fully separate itself from the egg shell died while hatching.

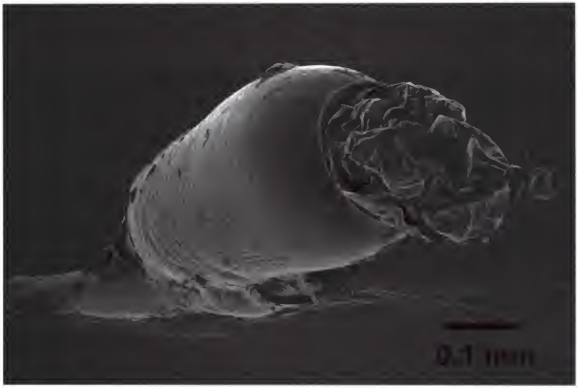

Fig. 2-21. Scanning electron micrograph of a head louse that died during hatch.

Fig. 2-22. Scanning electron micrograph of two 1st instar head louse nymphs.

Fig. 2-23. Scanning electron micrograph of a 2nd instar head louse nymph.

Fig. 2-24. Scanning electron micrograph of a 3rd instar head louse nymph.

Fig. 2-25. A scanning electron micrograph of an adult male head louse.

Fig. 2-26. A scanning electron micrograph of an adult female head louse.

CHAPTER 3
SEPARATION OF THE BODY LOUSE AND THE HEAD LOUSE THROUGH ANALYSIS OF CUTICULAR HYDROCARBONS

Introduction

In 1758 Linnaeus named the human louse, *Pediculus humanus*, providing no distinction between those populations of lice which inhabit the heads or those which inhabit the body and/or clothes of the host (Linnaeus 1758). Since that time many binomials and trinomials (Ferris 1951, Kim et al 1986) have been ascribed to what are commonly referred to as head lice and body lice.

Morphological characters are of little value when comparing head lice and body lice because many morphological characters shared by both overlap in size range (Buxton 1939, Ferris 1951). Early workers (Bacot 1917, Nuttall 1917b, 1919) found that the two forms were at times indistinguishable and could be forced to interbreed in the laboratory to produce viable offspring and so concluded that the two forms of lice were indeed one species or at most, subspecies. A more recent study of hosts with double infestations (Busvine 1978) showed morphological distinctions exist (overall body length and length of middle tibia) between the two species. Schaefer (1978) reviewed the data from Busvine and concluded that additional ecological separation exists between the two species in nature in terms of preferred habitat (head versus body) and micro-environment. In addition, Chirov and Ozerova (1997) concur with Busvine and Schaefer in separating the two species of lice based on biological and environmental factors.

Resolution of the controversy surrounding separation of head lice from body lice seems to be making progress. In this paper I present additional evidence from cuticular hydrocarbon analyses to make a distinction between the human head louse, *Pediculus capitis* DeGeer and the human body louse, *Pediculus humanus* Linnaeus.

Materials and Methods

Specimens. Head lice were obtained from consenting school children in Alachua County Schools, Florida and whose parents provided permission for the children to participate in head lice research conducted at the University of Florida. A local school nurse initially identified infested children and obtained permission for UF researchers to inspect infested children. The author was immediately notified and proceeded to inspect the children and collect head lice specimens. Soft touch forceps or fingers were used to remove live specimens from infested children. Lice were placed into 25 ml glass scintillation vials and killed by starvation at room temperature. Dried specimens were held in vials at room temperature until analysis. Specimens were collected on 04/05/99 (sample C), 10/14/99 (sample B), and 06/02/00 (sample A). Each sample was collected from children attending a different school. Sample A was comprised of 6 specimens from a single host. Sample B was comprised of 7 specimens from one host and 5 specimens from a second host. Sample C was comprised of 7 specimens from a single host.

Body lice were obtained from Insect Control Research Inc. (Baltimore, MD) which maintains one of the two laboratory reared colonies of body lice known in the world. This colony is the same colony from which much of the early research on body

lice was conducted (Culpepper 1940). Approximately 50 specimens (25 female and 25 male) were removed from host animals and placed into two 40-ml plastic vials separating males from females. Vials containing specimens were sealed and mailed to the Entomology and Nematology Department, University of Florida. Dried specimens arrived in Gainesville and were transferred to glass scintillation vials and stored at room temperature until analysis.

Cuticular hydrocarbon collection. Due to the relatively small number of specimens available, a technique was developed in which cuticular hydrocarbons could be collected from a single specimen. Miniature glass vessels were custom fashioned from standard disposable Pasteur pipets (FisherBrand, Pittsburgh, PA). The small tapered end of a glass pipet was broken into two pieces. One length (approximately 25 mm) was saved while the other much longer portion was discarded. The smaller diameter end of the 25 mm piece of tapered glass tubing was then held over a flame and rotated so that the end melted together forming a solid seal. This resulted in a miniature glass vessel (~25 mm long by ~1 mm diameter). The vessel could hold one individual louse specimen fully submerged in 8 µl of solvent. Each vessel was pre-rinsed with iso-octane to remove contaminants. A vessel was crafted for each individual specimen; no vessels were reused. Upon completion of hydrocarbon analysis, each specimen was sealed in its vessel with parafilm (American Can Co., Greenwich, CT), labeled and stored for permanent record at the University of Florida Entomology and Nematology Department.

Cuticular hydrocarbon extraction. An individual specimen was removed from its storage container and placed into one of the glass vessels with forceps and 8 µl of iso-

octane (2,2,4-trimethylpentane, Fisher Scientific 0299-1, Fair Lawn, NJ) added. After 10 min, a 10 µl microsyringe (model 80330, Hamilton Co. Reno, NV) was used to obtain a 2 µl sample of the solvent rinse after pulling approximately 3-4 µl of the solvent into the micro pipette and then exiting it 2 or 3 times. This technique ensured thorough rinsing of the specimen and subsequent collection of a suitable sample.

A 2 µl sample was injected into a Shimadzu (Kyoto, Japan) CC-14A AT-1 Heliflex, gas chromatograph equipped with a flame ionization detector. The gas chromatograph was set for splitless injection with an injection port purge after 30 sec. The gas carrier was helium with a flow velocity of 13.31 cm/sec. Initial column temp. was set at 200°C with a temperature rise of 4° C/min for 30 min. The column was a nonpolar fused silica capillary column coated with bonded polydimethylsiloxane RSL-150 (25 m, 0.25 mm, 0.2 m film) (Alltech, Deerfield, IL). Data were recorded directly from the gas chromatograph by a Hewlett-Packard 3390A integrator.

A standard sample (Sigma, St. Louis, MO) containing known hydrocarbons was used to compare with specimen samples. The standard sample contained all even and odd straight-chain saturated hydrocarbons with the following number of carbons: 20-30, 32, and 34. A 1 µl sample of the standard was analyzed to provide a standard chromatogram. The standard hydrocarbon sample was analyzed approximately once for every 10 specimen samples. Area under each peak was recorded electronically and relative percent calculated based on the total area under all peaks.

Data Analysis. Retention times of all peaks were converted into Retention Indexes (RI) using the following formula (Ettre 1964):

$$RI = 100n + \{100 \times \{(Rt\ unknown) - (Rt\ standard_n)\}/\{(Rt\ standard_{n+1}) - (Rt\ standard_n)\}\}$$

where; RI = retention index, Rt = retention time, n = number of carbons in a standard hydrocarbon, and n + 1 is a hydrocarbon with one additional carbon. A linear relationship between retention time as a function of carbon number was verified by a graphical plot (not shown). Retention indexes were calculated for all peaks. In addition, chromatograms were placed onto a light table and compared to standard chromatograms to aid in identification of saturated straight-chain compounds.

Individual hydrocarbon peaks were tabulated by the retention time of the peak and by comparison to the standard sample. Area under each peak was recorded electronically and relative percent calculated based on total area under all peaks. Mean relative percent of sample peaks common to all specimens was compared by Student's *t*-test ($P < 0.05$) (SAS 1990). Canonical discrimination analysis (SAS 1990) was used to compare relative patterns of peaks between head lice and body lice.

Results

Many body lice specimens were rendered useless during trial and error exercises to determine the appropriate quantity of solvent to bathe specimens in and the proper time allowed to soak before injection in order to provide an appropriate chromatogram. Once the proper method was developed a total of 21 body lice specimens (10♀ and 11♂) and 25 head lice specimens (17♀ and 8♂) were analyzed and provided appropriate records. A total of 32 hydrocarbon peaks was tabulated from the samples (Table 3-1). Not all peaks appeared in all samples but typical chromatograms of a head louse and body louse are provided in Fig. 3-1. Six hydrocarbon peaks with retention indexes 2200, 2336, 2406, 2962, 2973, and 3034 were unique to the body louse while only 3 peaks,

2507, 2744, and 2811 were unique to the head louse. There were 4 principal peaks present in all samples, 2500, 2700, 2900, and 3075. These four peaks represent approximately 50% and 75% of the cuticular hydrocarbons present on head lice and body lice, respectively, and were readily apparent on all chromatograms. The hydrocarbon with a RI of 3082 was an additional significant component in the head lice samples comprising 16% of the total area but was present in only 23 of the 25 samples.

Although no attempt was made to quantitatively compare cuticular hydrocarbons between males and females of either species due to relatively small sample sizes, there was a distinct qualitative difference between males and females of the body lice samples. RI 2962 was present in all female body lice but not in male body lice, and RI 2973 was present in all male body lice but not in female body lice. Neither 2962 or 2973 was present in any head lice samples.

Peaks 2500, 2700, 2900, and 3075 were present in every sample. Because these hydrocarbons are consistent and seem to be highly conserved, analyses were conducted by standardizing the overall percent composition using only these four peaks (Table 3-2). Mean relative percent of all four principal peaks was significantly different for head lice vs. body lice, 2500 (34.75% vs. 17.05% ; $t = 8.2458$, df = 44, $P < 0.0001$), 2700 (32.61% vs. 21.22%; $t = 8.3550$, df = 44, $P < 0.0001$), 2900 (22.35% vs. 43.06%, $t = 7.0818$, df = 44, $P < 0.0001$), and 3075 (10.24% vs. 18.65%, $t = 2.4809$, df = 44, $P < 0.0170$).

The same 4 principal peaks were analyzed by canonical discriminant analysis. A graphical representation of this analysis appears in Fig. 3-2. A separation of body lice from the three populations of head lice (A, B, and C) as a whole is clearly apparent along

the first canonical axis (x-axis). The body lice specimens clustered on the right side of axis 1 while all of the head lice specimens clustered on the left side of axis 1.

The standardized canonical coefficients, along with supplementary univariate tests, indicate that of the four peaks analyzed, peak 2900 was the major contributor to the separation of head lice and body lice ($F = 32.65$, $df = 3, 42$, $P < 0.0001$, $R^2 = 0.70$). The first canonical correlation provided the highest Proportion value (the proportion of measurable difference between the populations that is explained by the first canonical correlation) of 0.97 ($F = 14.42$, $df = 12, 103.5$, $P < 0.0001$) corresponding to the clear separation of head lice and body lice on the first canonical axis.

Discussion

Hydrocarbons are a major component of cuticular lipids found on the exoskeleton of insects (Lockey1988). Hydrocarbons are functionally important (Howard and Blomquist 1982, Lockey 1988) and can be unique at the species and even population level (Carlson and Service 1980, Haverty et al. 1996, Broza et al. 2000). Various insect taxa including crickets (Castner and Nation 1986), mosquitoes (Carlson and Service 1980, Kruger and Pappas 1993), termites (Haverty et al. 1996), bark beetles (Page et al. 1990), chrysomelid beetles (Golden et al. 1992), grasshoppers (Sutton et al. 1996) and honey bees (Carlson and Bolten 1984) can be discriminated by cuticular hydrocarbon composition. To our knowledge this paper represents the first attempt at analyzing cuticular hydrocarbons from human lice.

Although the cuticular hydrocarbon patterns provided in Fig. 1 do not quickly allow one to visually discriminate between the two species, statistical analyses of percent

composition of major peaks and canonical analysis (Figure 3-2) make it clear that the two are quantitatively different relative to hydrocarbon pattern. The fact that there are quantitative differences among the four principal peaks, 2500, 2700, 2900, and 3075 should allow a researcher to ascertain the identity of a specimen provided it has been properly preserved.

De Geer (1778) described the head louse as *Pediculus humanus capitis* and the body louse as *Pediculus humanus corporis*. At least a dozen trinomials have been used to describe various populations of head or body lice based on difference in host race or pigmentation of the specimen (Farrenholz 1917). After a thorough review, Ferris (1935) combined these into one species. Bacot (1917) conducted many studies on the biology of the two forms of the species and found slight differences in survival, oviposition rates, and morphological features. Bacot also found that mated head and body lice could produce viable offspring, suggesting that the two forms are of the same species. Keilin and Nuttall (1919) later pointed out that many of the hybrid specimens which Bacot produced were in fact hermaphroditic. Busvine (1948) reviewed all the available evidence at the time and believed that there was not sufficient evidence to promote the two types to separate species status and concluded that they are subspecies. After another review of the evidence, Ferris (1951) stated that a single species name, *Pediculus humanus* Linnaeus, was sufficient and that use of the common names "head louse" and "body louse" was up to the researcher when he/she thought it necessary.

The taxonomic status of *Pediculus humanus* was relatively stable until Busvine (1978) provided new evidence to separate the two forms. While investigating populations of head lice and body lice, Busvine found that the two forms retained their

characteristic size differences even though they inhabited the same exact host. In addition, no intermediate sized individuals were ever discovered. This new evidence along with the behavioral ecology exhibited by these two forms led Busvine to declare them to be two separate species, *Pediculus capitis* and *Pediculus humanus*. Schaefer (1978) also analyzed the data from Busvine (1978) and compared ecological factors. He concluded that the two species exist in two different environments which produce different selection pressures maintaining the different forms of lice.

Apparently, many researchers do not agree with Busvine (1978) and Schaefer (1978) as many continue to use the trinomial (Zeichner 1999, Mumcuoglu et al 1990, Downs et al 1999, Price and Benitez 1989, Pittman et al 1987, Urcuyo 1986, Gratz 1985, Alexander 1984) or variety (Taplin et al 1982, Taplin et al 1986, Pray 1989, Chosidow et al 1994) classifications. More recently Chirov and Ozerova (1997) reviewed the situation including the data contained within Busvine (1978) and Schaefer (1978) and again concluded that there was enough biological evidence to support the concept of two species. Interestingly, the editorial committee of the journal agreed with Chirov and Ozerova in elevating head lice and body lice to species level but disagreed with the authors on which scientific names are appropriate for each because of priority.

We believe that the cuticular hydrocarbon analysis reported here is further evidence to support Busvine's original position that the human head louse, *Pediculus capitis* deGeer is a distinct species from the human body louse, *Pediculus humanus* L. and identification can be made based on the composition of cuticular hydrocarbons of a specimen.

Table 3-1. Percent of 32 hydrocarbon peaks present in gas chromatograms from extracts of *Pediculus capitis* and *Pediculus humanus*.

	P. capitis (n = 25)		*P. humanus* (n = 21)	
RI	Mean % ± SD	n^a	Mean % SD	n^a
2008	–	0	0.27 ± 0.01	2
2176	4.16 --	1	1.34 ± 0.46	6
2200	–	0	0.36 ± 0.09	5
2300	3.03 ± 1.57	23	1.91 ± 0.70	18
2336	–	0	0.34 ± 0.09	4
2400	1.05 ± 0.64	16	0.92 ± 0.28	14
2406	–	0	0.67 ± 0.01	2
2500	17.87 ± 4.00	25	12.21 ± 4.70	21
2507	3.26 ± 0.63	3	–	0
2532	1.59 ± 0.58	19	0.63 ± 0.15	5
2600	1.49 ± 0.66	21	1.05 ± 0.29	15
2673	1.10 ± 0.71	14	0.49 ± 0.32	7
2700	16.90 ± 3.96	25	16.04 ± 4.34	21
2734	4.25 ± 1.14	22	0.87 ± 0.22	7
2744	1.28 ± 0.49	13	–	0
2800	1.43 ± 0.71	20	3.12 ± 1.62	21
2811	4.11 ± 2.61	21	–	0
2875	3.99 ± 3.22	21	4.65 ± 2.49	21
2900	11.32 ± 3.19	25	33.68 ± 10.69	21
2935	6.10 ± 1.68	22	4.96 ± 2.49	13
2943	2.11 ± 0.73	15	1.76 ± 0.38	7
2953	0.66 ± 0.34	9	0.81 ± 0.11	7
2962	–	0	0.97 ± 0.43	10
2973	–	0	1.54 ± 0.37	11
3000	2.50 --	1	0.31 ± 0.11	5
3034	–	0	1.08 ± 0.26	6
3075	4.23 ± 3.37	25	13.09 ± 7.71	21
3082	16.00 ± 6.48	23	6.11 ± 4.60	7
3100	2.91 ± 0.44	4	1.86 ±0.99	21
3132	6.15 ± 2.22	23	3.67 ± 1.50	13
3162	1.37 ± 0.80	15	2.36 ± 0.72	9
3322	2.39 ± 0.85	17	1.17 ± 0.17	5

[a] Number of specimens with peak

Table 3-2. Relative percent of cuticular hydrocarbons common to all specimens of head lice and body lice.

	P. capitis (n = 25)	*P. humanus* (n = 21)
RI	Mean % ± SD	Mean % ± SD
2500	34.75 ± 7.85a	17.05 ± 6.46b
2700	32.61 ± 5.19a	21.22 ± 3.78b
2900	22.35 ± 8.34a	43.06 ± 11.46b
3075	10.24 ± 10.93a	18.65 ± 12.04b

Means in rows followed by the same letter are not significantly different ($P > 0.05$; Student's *t*-test [SAS Institute 1990]).

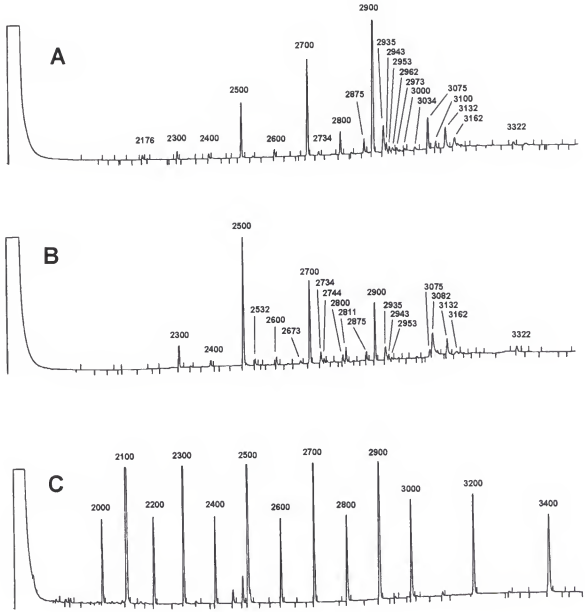

Fig. 3-1. Representative chromatograms of *Pediculus humanus* (A), *Pediculus capitis* (B), and commercial standard (C) containing known hydrocarbons. Peaks are identified by the corresponding Retention Index.

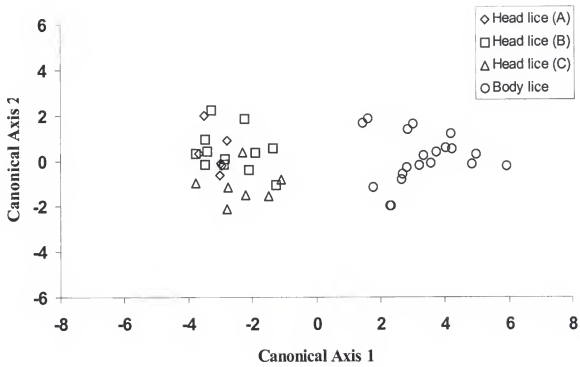

Fig. 3-2. Plot of canonical discriminant analysis utilizing the four primary peaks common to all body lice specimens and the three samples of head lice specimens.

CHAPTER 4
OFF-HOST SURVIVAL OF THE HUMAN HEAD LOUSE

Introduction

Infestations of head lice, *Pediculus capitis* DeGeer, are a major concern in elementary aged school children. Head lice have become more common in elementary schools than all other childhood communicable diseases combined (Donnelly et al. 1991). Because head lice are mostly transmitted by direct head-to-head contact (Slonka 1977, Chunge et al. 1991) most schools have policies which keep infested children out of school until they are lice-free (Altschuler and Kenney 1986, Gratz 1997).

In addition, many parents and school officials become concerned with the possibility that head lice will be transmitted from hats, desks, seats, and carpet (Altschuler and Kenney 1984, Clore 1988). Sometimes the classroom (or entire school) in which the infested child was present may be scheduled for insecticide treatment out of fear that some individual head lice or eggs may have fallen off the infested child and be transmitted to additional children. Many common recommendations for treatment of head lice state that head lice can survive off the human host no longer than 24 - 48 hrs (Juranek 1985, Burgess 1995) and that head lice eggs most likely will not hatch off-host (Maunder 1977) indicating that no treatment of the environment is necessary. There is surprisingly little in the literature to support these claims, most relying on the early observations of Nuttall (1917b) and Buxton (1939).

These early studies represent rough estimates, some of which were made using body lice. It was the purpose of this study to determine a more accurate representation of the survival of the head louse upon removal from its host, particularly as it would relate to indoor environments. Results should aid in head lice management programs at schools.

Materials and Methods

Specimens were collected from infested school children attending an Alachua County elementary school. Consent was provided by the child and by the parent of the child to inspect for head lice. Most specimens were collected during routine head lice screening initiatives. Soft touch forceps or fingers were used to remove live adult specimens from infested children. A specially designed nit comb was used to remove lice eggs. Adult lice and eggs were placed into separate 25 ml glass scintillation vials and returned to the laboratory.

A total of 300 viable eggs were used during experiments. Lice eggs were examined with a dissecting microscope to determine viability. Eggs which appeared to be living and possessed an intact operculum were defined as viable. Ten viable eggs were placed into a 15 x 100 mm plastic petri dish containing a 75 mm dia piece of Whatman paper. Three Petri dishes were prepared in this manner and each placed into an environmental chamber set at a temperature of 20°, 25°, or 30° C. Relative humidity for all environmental chambers was set at 60% RH. Egg hatch was recorded daily for 30 days. Only nymphs able to fully escape from the egg shell were recorded as successfully hatching. This process was replicated 10 times. These temperatures were chosen

because 20° and 25° C represent the range of room temperatures which fallen head lice would be exposed and 30° C represents the temperature of the preferred environment of the head louse, the human head (Busvine 1948).

A total of 234 adult lice were used during experiments. Adult lice were removed from collection vials and placed onto the back of the author's hand for 10 minutes to allow for a final blood meal before initiation of experiment. Upon termination of feeding, lice were placed into plastic Petri dishes (15 x 100 mm) containing a 75 mm dia piece of Whatman paper (No. 1). Initial collection of lice was split into three equal sized groups ranging from 7 to 10 specimens for each treatment. Petri dishes containing lice were placed into environmental chambers and reared as described above. Mortality was recorded every 4 hours until 100% mortality.

Data analysis. Mean percent mortality of adult lice was analyzed using probit analysis to obtain LT_{50}'s (SAS 1990). Percent egg hatch was submitted to analysis of variance with Duncan's multiple range test ($P = 0.05$) employed to separate means (SAS 1990) and days to egg hatch separated with Student's *t*-test (SAS 1990).

Results

Overall off-host survival of adult head lice is represented in Fig. 4-1 with results of probit analysis presented in Table 4-1. The LT_{50} values (95% CI) were 24.8 (23.4 - 26.3), 21.2 (19.7 - 23.0), and 14.2 (13.3 - 15.3) for 20°, 25° and 30° C, respectively. No lice survived beyond 48 hrs off-host at any temperature. At 30° C, no lice survived beyond 28 hrs.

Temperature had a significant effect on egg hatch (Fig. 4-2). The mean percent egg hatch was 0 % at 20° C, 24% at 25° C, and 82% at 30° C ($F = 133.3$; $df = 2, 27$; $P = 0.0001$). Analyzing those eggs which successfully hatched using Student's *t*-test revealed a difference in mean days to egg hatch (Fig. 4-3). The mean days to egg hatch for eggs exposed to 25° C was 3.2 while at 30° C, the mean days to egg hatch was 4.4 ($t = -2.104$; $df = 104$; $P = 0.0378$).

Discussion

Nuttall (1917b) first described the greatest number of days which the human head louse could survive away from its host as 30 hrs at 33° C, 47 hrs at 30° C, 96 hrs at 20° C and 143 hrs at 12° C . Only a few individual lice were used for each experiment and the relative humidity to which these lice were exposed is unclear. Buxton (1939) conducted similar experiments on body lice (*Pediculus humanus* L.) and determined that the extreme length of life for recently fed then starved body lice was 2 days at 30° C, 4 - 5 days at 20 - 30° C, 7 days at 10 - 20° C, and 7 - 9 days at 0 - 10° C. It is expected that body lice would exhibit different survival characteristics because the biology of the body louse is different from that of the head louse (Nuttall 1917b, Bacot 1917, Buxton 1939, Busvine 1948).

While comparing many biological and morphological characters of the two species of *Pediculus*, Busvine (1948) recorded the off-host survival of recently fed head lice. He recorded 0% and 100% mortality for males and females respectively at 24 hrs. when reared at 30° C. On his next observation at 41 hrs, 100% of males were dead. At 23° C, 100% mortality was first recorded at 72 hrs for females and 96 hrs for males. All

of these experiments were conducted under 75% relative humidity but observations on mortality were made at irregular periods of time.

My results indicate a shorter survival time for adult head lice than that previously reported by Nuttall (1917b) and Busvine (1948). One explanation may be the difference in relative humidity although it has been demonstrated that relative humidity has little impact on survival of head lice except at very low or very high levels (Lang 1975). Lang (1975) reported a mean survival time of 24 hrs (sd = 1.8) for head lice reared at 26 ± 2° C (RH = 75%) and a mean of 8.8 hrs (sd = 1.8) for head lice reared at 36 ± 2° C (RH = 75%). These results more closely resemble the findings of the current study.

The incubation period for eggs of body lice has been recorded as between 7 and 11 days with a mean of 8.5 days when reared at 30° C in a "humid" environment (Bacot 1917). Nuttall (1917b) observed a longer range for body lice eggs reared at 30° C indicating that 11 - 13 days was most common. In a comprehensive study Leeson (1940) found the lower limit for successful hatch of body lice eggs to be 24° C and the highest limit to be 37° C when constantly reared at these temperatures. Leeson also discovered that temperature the range of 29 - 32° C produced the highest successful hatching rate (97%) with an incubation period of 7 - 11 days. Leeson also found that relative humidity did not significantly affect the incubation period but did influence hatch rate, however, only at very low or very high levels of relative humidity.

After rearing head lice eggs in a special container strapped in various locations on the human body (ranging in temperature from 30 - 35° C) Nuttall (1917b) reported a range of 6 - 10 days with the majority hatching on day 7. Busvine (1948) reported similar results (rearing at 30° C and 75% RH) adding that body lice and head lice exhibit

very similar egg hatching periods in the laboratory with the exception that body lice exhibit greater hatching rates; 97% and 84% for the body louse and head louse respectively.

My results indicate the mean hatching rate for head lice eggs reared at 20°, 25°, and 30° C (60% RH) to be 0%, 24% and 82% respectively. This compares well with Leeson's (1942) observations on body lice eggs and Busvine's report (1948) on head lice eggs as described above. However our mean incubation periods of 3.2 and 4.4 days for 25° and 30° C respectively are considerably different. This likely is due to the fact that the eggs used in the current study were of mixed development. No effort was made to ascertain the stage of development of the embryonic head louse. However, a batch of viable head lice eggs in random stages of development reared at 30° C (preferred environment) would provide a mean hatching period of about 4 - 5 days. This would indicate that the eggs used in the current study were in various stages of development but represented equally.

The incubation period for eggs reared at 25° C was significantly shorter, 3.2 days, probably because of two factors. The first factor being that fewer eggs hatched. Secondly, the only eggs that hatched were most likely the eggs which had reached a certain stage of development and therefore could complete development at the lower temperature. Leeson (1941) made similar observations when removing mature body lice eggs from a preferred temperature and placing them in a cooler temperature.

First Buxton (1939) then Juranek (1977) indicated increased research into the off-host survival and potential egg hatch of head lice was vitally important to improve

control programs for pediculosis outbreaks. It is my hope that these results contribute to the knowledge of the biology of head lice.

This information can be used to enhance current head lice management programs. Because adult lice can live no longer than 48 hrs at room temperature, a weekend serves as a good "treatment" to kill any crawling lice which may have become dislodged from an infested child at school. This may or may not satisfy all school principals or parents, however, because any dislodged head lice eggs likely would survive such a "treatment", hatching up to 9 days after being removed from the host. Although the probability of a recently hatched head louse nymph being successful in infesting a new host before dying is most likely very small, the potential remains. This would have to occur after a viable head louse egg were to become dislodged from an infested child's head. Considering that children in the lower grades (who happen to be the most infested) spend considerable time sleeping and laying on the carpet of their classroom or sitting on the carpet during reading time (for example), this mechanism of transfer does not seem impossible.

Although Maunder (1977) suggests that very little if any potential exists for fallen head lice or dislodged head lice eggs to result in additional infestations, our research suggests that this mechanism should not be overlooked. Currently, most schools utilize a policy which removes infested children immediately upon being identified in order to prevent transmission (Altschuler 1998). We suggest that classrooms identified as having had an infested child present, should be vacuumed at the very least, once the child has been removed. The most practical would probably be to vacuum the classroom at the end of the school day. This would presumably remove any dislodged head lice or eggs.

Table 4-1. Influence of temperature on time to mortality for recently fed head lice adults upon removal from host.

Temp (°C)	*n*	Slope ± SE	χ^2	LT_{50} (hrs)	95% C.I.	Max.(hrs)[a]
20	77	5.60 (0.68)	1.90	24.8	23.4 - 26.3	48
25	79	4.23 (0.54)	2.34	21.2	19.7 - 23.0	40
30	78	5.37 (0.60)	1.37	14.2	13.3 - 15.3	28

[a] Represents time to 100% mortality.

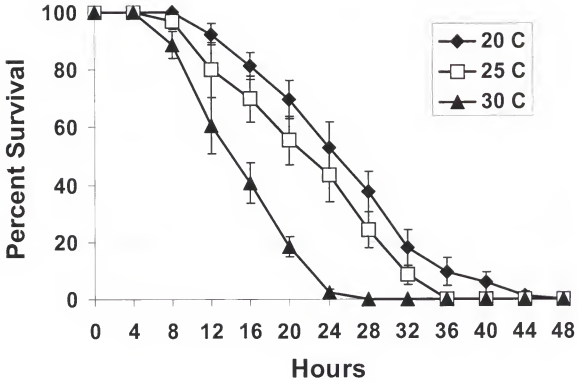

Fig. 4-1. Off-host survival of recently fed adults of the human head louse held at three different temperatures and 60% RH. Error bars represent 95% C.I.

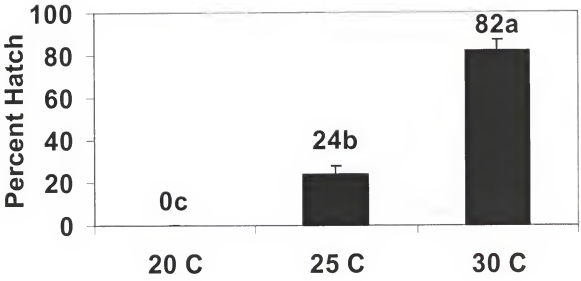

Figure 4-2. Influence of temperature on mean percent hatch of head lice eggs held at 60% RH. Error bars represent 95% C.I. Means followed by the same letter are not significantly different ($P > 0.05$; Duncan's Multiple Range test [SAS Institute 1990]).

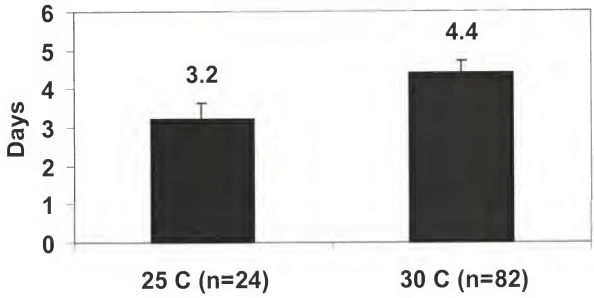

Figure 4-3. Mean days to hatch for head lice eggs held at two temperatures and 60% RH. Error bars represent 95% C.I. Means are significantly different ($P < 0.05$; Student's *t*-test, [SAS Institute]).

CHAPTER 5
COMPARISON OF CURRENT CONTROL PRACTICES FOR THE HUMAN HEAD LOUSE AND OBSERVATIONS ON THE EPIDEMIOLOGY OF CHRONIC CASES OF PEDICULOSIS

Introduction

Head lice, *Pediculus capitis* De Geer, have become increasingly pestiferous among school children in recent years (Downs et al. 1999a). It is estimated that 2.6 million households experience head lice outbreaks (Andrews 1992) resulting in approximately 6 to12 million children per year becoming infested with head lice in the United States annually (Clore and Longyear 1990). Over the past 15 years pyrethroid-containing products have become the most preferred products among parents for treatment of pediculosis (Burgess 1995, Meinking 1999). Recent reports stated that wild head lice populations exhibit decreased levels of susceptibility to many pediculicides including pyrethroid insecticides (Chosidow et al. 1994, Downs et al. 1998, Pollack et al. 1999) and even demonstrated pyrethroid resistance in head lice (Mumcuoglu et al. 1995, Picollo et al. 2000) indicating possible cause for the increase in head lice problems.

Synergists are often added to insecticide formulations to increase toxicity of active ingredients and improve efficacy (Wilson 1949). A popular pediculicide product available in the United States contains piperonyl butoxide (PBO) to synergize the activity of the pyrethrin active ingredient.

It has been suggested that insecticide resistance is not solely to blame for the increase in head lice infestations and that improper treatment (Goldsmid 1990, Burgess et al. 1995, Meinking 1999) or misdiagnosis (Pollack et al. 2000) is often related to presumed product failure. It is the purpose of this study to compare the effectiveness of the two most widely used active ingredients (permethrin and pyrethrin synergized with PBO) in the United States against head lice. In order to control for user error, all treatments were applied using manufacturer's directions and under the supervision of the author.

Materials and Methods

All field work was conducted in Alachua County Schools, Florida. Children infested with head lice were initially identified by a school nurse and retained in the school's clinic. Participants in the study were not randomly selected during school-wide screening procedures. All children participating in the study were identified by the school nurse or the parent as having a chronic case of head lice persisting after various treatments applied by the parent resulted in "failure". Most research participants had been battling head lice for several months. After consent was provided by the child and the child's parent or guardian a closer inspection was conducted to estimate number of viable eggs, nymphs, and adults of head lice. In addition, basic demographic data was recorded for each child. Upon completion of inspection one of two treatments was randomly selected (coin toss). The two possible treatments were a permethrin (1%) containing creme rinse (Nix®) and a pyrethrin (0.33%) and piperonyl butoxide(4.0%) containing shampoo (Rid®). Products were purchased from local retail stores and

expiration dates and lot numbers recorded. A total of 52 children were treated, 23 with Nix and 29 with Rid.

Products were applied by the school nurse closely following all product label directions. Children were escorted to the bathroom and their hair wetted using a shower head. In the case of a Nix treatment, the child's hair was washed using a basic store brand shampoo before treatment with the pediculicide. Products were according to label directions and allowed to remain for 10 min. only. After 10 min., the child's head was completely rinsed with water. A white towel was used to lightly dry the child's hair and the child was asked to remain sitting in a chair for 15 min. During this 15 min. the white towel was placed on a table and any lice (live or dead) observed were removed and placed into a 25 ml glass scintillation vial using a pair of feather weight forceps or fingers.

After 15 min. the child was again inspected and all visible lice removed using feather weight forceps and placed into a 25 ml glass scintillation vial. At this point the child's hair was properly groomed and the child allowed to return to class or taken home by the parent, depending on the individual school's policy. All lice were taken back to the laboratory for further evaluation.

A small number of children (7), receiving no treatment, were examined and head lice removed from their heads were used as a control. These lice were removed and stored using the same procedure as described for the treated samples.

Upon arrival at the laboratory, lice were removed from the scintillation vial and placed into a 15 x 100 mm plastic petri dish containing a piece of Whatman paper (No. 1). Petri dishes containing lice were immediately placed into an environmental chamber

and held for 4 hrs at a temperature of 25° C and RH of 60%. Mortality was recorded at 4 hrs and 24 hrs post-treatment for all lice. Lice were considered dead if after being placed on back of the author's hand they did not feed or did not crawl after 5 min.

Data analysis. Comparison of treatments and demographic data of research participants was analyzed by Chi-square analysis or Fisher's Exact test (SAS 1990). Mean percent mortality of head lice collected from treated children was ranked separately at 4 hrs and 24 hrs post-treatment (Conover and Iman 1981). Ranks were submitted to analysis of variance (SAS 1990), and means were separated by Duncan's multiple range test ($P = 0.05$; SAS Institute 1990).

Results

There were no statistical differences among the two treatment groups with regard to various demographic and physical characteristics (data not shown). Demographic data of all participants is presented in Table 5-1. Within the entire sample of chronically infested children, 4th grade was significantly over represented ($\chi^2 = 30.38$, df = 6, $P = 0.001$) as were females ($\chi^2 = 22.23$, df = 1, $P = 0.001$), straight hair ($\chi^2 = 7.69$, df = 1, $P = 0.006$), and brown hair ($\chi^2 = 29.69$, df = 3, $P = 0.001$). However, these characteristics taken together (4th grade females, with straight, brown hair) accounted for only 9 of the 52 (17.3%) chronically infested children.

Estimated infestation rates (Table 5-2) were identical among treatment groups with the exception of viable nits ($\chi^2 = 9.25$, df = 3, $P = 0.02$). This was mostly a result of none of the Nix-treated children having between 20 - 49 viable eggs. Because the value is marginal and based on a rough estimate, no relationship is expected between egg

infestation rate and treatment. In addition there is no known relationship between egg infestation rate and survival of lice.

Head lice nymphs were removed from 17 of 23 Nix-treated children and 26 of 29 Rid-treated children. Although great care was exercised in attempting to collect all lice, head lice nymphs are relatively small (particularly 1st instars) and it is possible that some specimens may have escaped collection. Head lice adults were removed from all 23 Nix-treated children and 27 of 29 Rid treated children. Numbers of total lice removed from children ranged from 5 to 128.

Results of treatments on mortality of adult lice is presented in Table 5-3. Rid and Nix caused greater initial mortality (4 hrs after treatment) compared to the untreated control ($F = 11.34$; $df = 2, 54$; $P = 0.0001$). At 24 hrs after treatment Nix and Rid provided equal mortality, 83.4% and 80.1% respectively, with only marginal significance relative to the control ($F = 3.32$; $df = 2, 54$; $P = 0.0437$).

Relative effectiveness (modeled success of treated children) of the two treatments was not different with regard to head lice nymphs at 4 hrs ($P = 0.513$, Fisher's Exact) or 24 hrs ($P = 0.114$, Fisher's Exact) after treatment (Table 5-4). Effectiveness was also not different among treatments with regard to head lice adults at 4 hrs ($P = 0.308$, Fisher's Exact) or 24 hrs ($P = 0.569$, Fisher's Exact) after treatment. The percent of samples with no live lice at 24 hrs after treatment (symbolizing a successful treatment) was 30.4 % and 48.3% ($P = 0.259$, Fisher's Exact) for Nix and Rid respectively.

Discussion

Using this method we attempted to simulate the conditions to which head lice would be exposed during a typical shampoo treatment following the manufacturer's directions. Head lice were exposed to the toxicant for exactly 10 min. followed by a thorough rinsing of the hair with clean water. Any toxicant remaining on a treated head subsequent to washing must be minimal as the manufacturer's directions indicate that human exposure should not exceed 10 min. Removing lice from a child's head following treatment should accurately model survival of head lice from such treatment. Results of the shampoo treatments in this study indicate that inadequate control was achieved with this method.

Pyrethroids have been shown to be ineffective against head lice in France (Chosidow et al. 1994), Israel (Mumcuoglu et al. 1995), and the UK (Downs et al. 1999c). One recent report (Pollack et al. 1999) indicated head lice in Florida (USA) are less susceptible to pyrethroids than populations of head lice found in Borneo where pyrethroid-containing products are not used.

Original research conducted with permethrin showed that greater than 99% of children treated with NIX were free of lice 7 days after treatment (Brandenburg 1986). Immersing head lice into NIX provided 100% mortality at 10 min. (Meinking and Taplin 1990). However, Mumcuoglu et al. (1995) showed a 4 fold decrease in susceptibility of head lice to permethrin from 1989 to 1994 with the LT_{50} value of 1% permethrin being approximately 5.2 hrs in field collected lice in 1994. Results of the current study support these findings and even indicate an increasing trend in the ability of head lice to survive permethrin treatments.

The pyrethrin and PBO -containing product, Rid, was tested in 1987 (Pitman et al. 1987) when it exhibited 60%, 75%, and 100% mortality against samples of head lice at 0 hrs, 2 hrs, and 24 hrs post-treatment respectively. Additionally, in vitro studies (Meinking et al. 1986) determined the mean time to 100% mortality for samples of field collected head lice exposed to Rid was 10.5 min (SD = 3.4). My results suggest a significant decrease in the ability of Rid to provide mortality in head lice compared to these previous reports.

In this study I report almost 70% of Nix-treated samples contained live head lice 24 hrs after treatment (Table 5-3) mirroring an 82% failure rate recorded for permethrin in Britain (Downs et al. 1998). Apparently, addition of a synergist did not improve treatment success as almost 52% of Rid (containing PBO)-treated samples contained live head lice 24 hrs after treatment. This represents a significant increase in the ability of head lice to survive such treatments compared to the original published data. These results support claims by Downs et al. (1999c) and Hemingway et al. (1999) and subsequently substantiated by Lee et al. (2000) that the mechanism of resistance to pyrethroids exhibited by head lice is related to nerve insensitivity (*kdr*).

There are products available in the U.S. which contain higher concentrations of permethrin (5% by prescription only); however, it is unclear whether use of these products would increase treatment success (Pollack et al. 1999). Some schools have relied on manual removal with special lice combs as the primary treatment method. Unfortunately, this method has shown to be ineffective (Downs et al. 2000b, Roberts et al. 2000). Research into new compounds for use in pediculicides is needed and may result in increased efficacy with "reduced risk" compounds (Downs et al. 2000a).

General infestation rates among females have previously been reported as higher than that of males among elementary school children (Slonka et al. 1976, Vermaak 1996, Downs et al. 1999b). Additionally there may be some evidence that children in the 4th grade are more likely to be infested with head lice (Price and Benitez 1989, Downs et al. 1999b, Speare et al. 1999). This is the first known report on the epidemiology of chronically infested children. Chronically infested children may act as the primary sources for transmission in schools. Results of the current study indicate that school health officials in Alachua County should pay close attention to 4th grade females during head lice checks to optimize efficiency in screening programs. However, additional studies would be necessary to confirm the same trend in other localities.

In addition, improved methods for determining efficacy of pediculicides may be necessary. Untreated controls in this study exhibited 55.2% mean mortality reared at 25° C for 24 hrs. Chosidow et al. (1994) reported even greater mortality (76.6%) of untreated head lice, after being exposed to 28° C for 24 hrs. When exposed to 36° C (approximately human body temperature) adult lice live only a mean of 8.8 hrs (Lang 1975). It is clear that the human head louse does not survive well off its host. Any in vitro pediculicidal tests attempting to model the environment to which lice would be exposed on a human head must take into account these factors, as recently recommended by Burkhart and Burkhart (2001).

Table 5-1. Demographic data for all chronically infested children.

Characteristic	Total number of children (%)
Grade*	
K	2 (3.8)
1	4 (7.7)
2	7 (13.5)
3	9 (17.3)
4	19 (36.5)
5	10 (19.2)
6	1 (1.9)
Gender*	
Female	43 (82.7)
Male	9 (17.3)
Length of hair	
Long	19 (36.5)
Medium	16 (30.8)
Short	17 (32.7)
Color of hair*	
Black	15 (28.8)
Blond	7 (13.5)
Brown	28 (53.8)
Red	2 (3.8)
Texture of hair*	
Curly	16 (30.8)
Straight	36 (69.2)

* Frequency of characteristics were significantly different ($P < 0.05$); Chi-Square test for equal proportions [SAS Institute 1990]).

Table 5-2. Infestation estimates for all treated children.

Stage of head lice	Number of Children (%)	
	Nix® (n = 23)	Rid® (n = 29)
Viable nits*		
< 20	8 (34.8)	13 (44.8)
20 - 49	0 (0)	5 (17.2)
50 - 100	6 (26.1)	1 (3.4)
>100	9 (39.1)	10 (34.5)
Live nymphs		
< 5	9 (39.1)	9 (31.0)
5 - 15	8 (34.8)	9 (31.0)
16 - 50	2 (8.7)	9 (31.0)
> 50	4 (17.4)	2 (6.9)
Live adults		
< 5	12 (52.2)	10 (34.5)
5 - 15	4 (17.4)	9 (31.0)
16 - 50	4 (17.4)	8 (27.6)
> 50	3 (13.0)	2 (6.9)

* Frequency of characteristics were significantly different ($P < 0.05$; Chi-Square test [SAS Institute 1990]).

Table 5-3. Response of adult head lice to two types of shampoo treatments.

Treatment	# children	# lice/child	% mortality (SE)[a] at time after treatment 4 hr	24 hr
NIX (Permethrin)	23	21.5 (4.9)	63.74 (6.30)b	83.39 (4.12)b
RID (Pyrethrins + PBO)	27	25.3 (5.6)	59.78 (5.73)b	80.11 (4.55)b
Untreated	7	11.3 (0.7)	0.0 (0)a	55.24 (9.24)a

[a]Means within a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's multiple range test [SAS Institute 1990]).

Table 5-4. Efficacy of head lice treatment to kill all lice.

Stage of development	Treatment	*n*	% of samples with no live lice[a] 4 hr	24 hr
Adults				
	NIX	23	13.0a	34.8a
	RID	27	25.9a	44.4a
Nymphs				
	NIX	17	23.5a	41.2a
	RID	26	34.62a	69.2a
Adults or Nymphs				
	NIX	23	–	30.4a
	RID	29	–	48.3a

[a]Mean percent of samples with live lice in a column followed by the same letter are not significantly different ($P > 0.05$; Fisher's Exact Test [SAS Institute 1990]).

CHAPTER 6
A MODEL FOR STATE-WIDE IMPLEMENTATION OF INTEGRATED PEST MANAGEMENT IN SCHOOLS

Introduction

In 1993 the National Academy of Sciences published a report indicating that children were at a higher risk to pesticide exposure than adults were because of a child's smaller body size, higher metabolism, developing immune systems, and playful behavior (NRC 1993). In response to this report and many other concerns, the United States Congress passed the Food Quality Protection Act (FQPA) in 1996. As directed by the FQPA the United States Environmental Protection Agency has been reviewing the registration of many pesticides in hopes of ensuring the safety of compounds which are used in homes and schools for pest control. Because children between the ages of 5 and 18 spend approximately 1/6 of their lives in schools, many schools have restricted the use of pesticides on school grounds (Owens and Feldman 1998) and beginning in the early to mid 1990's many school districts began considering implementing Integrated Pest Management (IPM) within their schools.

Although the term IPM is old in traditional agricultural arenas, it is relatively new to most indoor applications. The concept of preventing pests and managing pests through a variety of means and using pesticides judiciously was almost completely new to most individuals conducting pest management in schools.

Beginning in 1996 the Florida Department of Education and the University of Florida Department of Entomology and Nematology partnered to respond to parental concerns over pesticide use in schools. The result was the formation of the Florida School IPM Advisory Board (FSIPMAB). The ultimate goal of the FSIPMAB was to implement IPM within all schools state-wide through intense education and training of pest management personnel working in/for schools. This paper describes the result of five years of effort on behalf of the FSIPMAB and reports success and failures of a voluntary program.

Materials and Methods

Florida School IPM Advisory Board. The FSIPMAB was established in 1996. Members of the committee include representatives from the following organizations: University of Florida Departments of Entomology and Nematology, and the Pesticide Information Office; Florida Departments of Education, Agriculture and Consumer Services, and Health; the Legal Environmental Assistance Foundation; USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology; County Cooperative Extension Service; concerned citizens; Florida school districts; Florida Pest Control Association; and the Certified Pest Control Operators of Florida Association. All activity to implement IPM into Florida's schools has been a concerted effort from the entire FSIPMAB. The Board meets twice every year to review program efforts and address continuing issues.

Pre-Training Assessment. A survey instrument was created to help determine how pest control was currently being conducted in Florida's school districts. The survey

consisted of 14 questions, many of which contained multiple parts. The survey was designed to be completed by a school district employee who supervises pest control programs. The survey was first conducted in 1996 before any training occurred.

Several steps were taken to facilitate as high a response rate as possible with returned surveys. The survey was only one page, front and back with a total of 14 questions. Several questions were simple yes or no answer questions. Because some of the information requested could be perceived as sensitive, recipients were informed that their individual survey information would be kept confidential. Recipients were also informed that they would receive a copy of the survey results. The survey was distributed to appropriate personnel in all 67 school districts in Florida through the Florida Department of Education (DOE). I believe the involvement of DOE at this level was crucial in obtaining as high a response rate as possible. Respondents were asked to complete the survey and return to DOE. After the deadline for return had passed a single telephone contact was made to each survey recipient who had not yet returned the survey and an additional survey delivered to them if requested. Upon final deadline hard copies of all completed surveys were delivered to the University of Florida for analysis.

Training. In order to implement IPM statewide it was determined that several audiences would need to be targeted for education in IPM. At the local level, many school district employees rely on County Cooperative Extension Service agents for knowledge in pest management. In order for this mechanism to continue functioning additional training regarding School IPM was initiated for county extension agents in the form of a two day "In-Service" workshop. School employees and pest control operators would also require additional training so a series of workshops was created to cover

specific technical details of IPM. To facilitate continuing education on School IPM a World Wide Web Site was created specifically on School IPM. This web site contains information directed at everyone involved with School IPM from the parents to the pest management professional.

School IPM Workshops. In response to the preliminary results of the survey, an agenda was created for a workshop to focus on presumed weaknesses present in current pest management programs. Workshops targeted private pest control operators and in-house school employees conducting pest control, but many concerned parents and school administrators also attended. Specifically the agenda addressed: 1) introducing the concept of IPM, 2) presenting reasons to switch to IPM, 3) how to control the top three pests (cockroaches, ants, rodents) using IPM, 4) testimonials from school employees currently using IPM, 5) guidelines for contracting IPM, and 6) small group hands-on training in kitchen inspection, pest identification, monitoring, grounds pest control, and vertebrate pest control devices. The workshops were generally 6 hours long with 45 min. provided for lunch.

The workshop was presented at 5 different sites over three months across the state of Florida to facilitate participation from all interested individuals. Each workshop was held at a local County Cooperative Extension Office. Fortunately, all of the facilities contained kitchens and grounds areas similar to that found in schools, which benefitted the inspection training. The schedule of workshops was disseminated to school district employees through standing DOE mechanisms. The pest control industry was informed of the workshops through free advertisements placed in monthly trade magazines of two state pest control associations (*Advantage* and *PCO* magazines). Advertisements of the

workshops were donated by the pest control associations. County Cooperative Extension Service agents were informed through internal memos. The workshop topics were approved for state re-certification credit for those individuals possessing certified pest control operator's licenses. All attendees were asked to complete an evaluation of the workshop at the conclusion. This evaluation consisted of 13 questions asking the respondent to rate various aspects of the workshop on a scale of 1-5, with 5 being the highest.

In-Service Training for Extension Agents. A two-day workshop was held in Gainesville, Florida at the Entomology and Nematology Department for interested County Extension Agents. All counties were informed of the workshop and that enrollment was limited to 25 persons. The topics covered during the two days included 1) Introduction to School IPM, 2) general insect identification (using dissecting microscopes and taxonomic keys), 3) turf and ornamental pest management, 4) allergies associated with insects, 5) termite control, 6) IPM for cockroaches, ants, and stinging/biting insects, 7) pesticide safety, 8) contracting issues and record keeping, and 9) real-life experiences using IPM in schools. Speakers for this event included University of Florida Extension specialists, graduate students, USDA-ARS scientists, and DOE personnel. At the conclusion of the training, attendees were asked to evaluate the workshop. The evaluation consisted of 5 questions rating various aspects of the workshop and a 6th open-ended question soliciting any suggestions or comments. Funding for this workshop was provided by the Office of the Dean of Extension at the University of Florida.

World Wide Web Site development. In 1997 the FSIPMAB secured funding from Region 4 of the US EPA to develop a WWW site devoted to the subject of School IPM. The site was designed to be appealing to all stakeholders associated with School IPM and is structured in five basic sections; 1) What is IPM, including definitions and instructions on how to implement IPM in a local school whether you are a parent, pest control operator, or concerned school employee; 2) Administrator information section, including training information provided in downloadable PowerPoint™ (Microsoft Corporation, Seattle, WA) presentations, model workshop agendas, examples of communication forms and documents to be used in an IPM program; 3) Technical section, including identification and management recommendations on over 20 structural pests; 4) Teaching curriculum section, including examples of class lectures on IPM to be used by teachers in the classroom; and 5) Related links section, providing www links to local, state, and federal resources helpful to those individuals working in IPM. Although initially designed to be a regional resource, the site's immediate popularity lead to enhancements making it the National School IPM web site. The goal of the WWW site was to provide a lasting and updated resource to support IPM practitioners once the initial wave of training workshops were completed.

Post-Training Assessment. Upon completion of all training workshops a second survey was conducted among all 67 school districts in 1997 using the same instrument as in 1996 to assess any impact resulting from the IPM training. Again, surveys were distributed from the DOE to the school districts addressed to the supervisor of pest management. Recipients were given three weeks to reply and then delinquent counties were telephoned by DOE personnel and encouraged to respond to the survey. A third

survey was also conducted in 1999 to determine whether any change observed in the 1997 data remained even after no additional training workshops had taken place.

Data analysis. Results from surveys were sorted and organized by code. Because the response rate was not 100% it was treated as a sample of the total population of 67 to determine sampling error. The following formula was utilized to determine sampling error: $\{ \sqrt{(X \div T)} \times 1.96 \}$, where X = (response rate * (1 - response rate) and T = total number of surveys distributed. The constant 1.96 provides for a 95% confidence interval. Although the response rate varied year to year, only survey data from school districts responding to all three survey dates were analyzed.

Results and Discussion

School IPM Workshops. Regional workshops were held in Marianna (NW), Kissimmee (Central), Tampa (West-Central), Ft. Myers (SW), and Ft. Lauderdale (SE). Representatives from every school district had attended at least one of the workshops. There were between 100 and 200 participants at each workshop with total attendance of all five workshops being 689. Workshop topics were covered by 6 different speakers at each site including university extension specialists, graduate students, and DOE personnel. A summary of evaluations completed by the participants is provided in Table 6-1. On a scale of 1-5 with 5 being the highest level of satisfaction, all 13 categories of the workshop had a mean ranking of 4.36 or higher. The highest rated category was "course content" with a mean of 4.78.

In-Service Training. Training for county extension agents occurred over a three day period from March11-13 1997. Agents from 18 of Florida's 67 counties attended. Each day consisted of approximately 6 hours of classroom and laboratory instruction. Instructors included university extension specialists, USDA-ARS researchers, graduate students, DOE personnel, and school district employees. A summary of program evaluations completed by participants is provided in Table 6-2. Overall the participants enjoyed the workshop with 100% reporting they were at least "satisfied" with the training and 80% were "very satisfied". Open ended responses indicated that the workshop was among the best which this group of extension agents had attended with comments like "This is one of the best trainings I've had in 18 years of extension" being representative of the entire group.

WWW Site Development. The National School IPM WWW site (http://www.ifas.ufl.edu/~schoolipm/) initially consisted of materials designed and developed solely by University of Florida personnel. A color brochure was created describing the web site and over 40,000 copies distributed across the country through various Extension Service and US EPA personnel. With increased awareness of the site came increased popularity and increased interest in incorporating the work of collaborators. Ultimately the site has become a clearinghouse of information about all aspects of School IPM with over 20 contributors from across the US. As of the Fall of 2000 the National School IPM WWW Site receives between 200 and 250 hits per week.

Survey Results. Results of all three survey dates (1996, 1997, and 1999) appear in Table 6-3. The number of surveys returned out of a possible 67 were 60, 59, and 57

for the years 1996, 1997, and 1999 respectively. All data presented is based on the 50 school districts (75% response rate) which returned surveys for all three years.

According to the 1996 survey, the top two pests in schools were cockroaches and ants (Fig. 6-1). Between 1996 and 1999 ants replaced cockroaches as the top pest. This probably was a function of both IPM adoption and technology. A primary component of IPM is targeting the pest insect. Although most of the indoor applications of sprays were targeting cockroaches, some control of ants was most likely occurring. As more schools adopted IPM, use of sprays decreased and use of bait formulations increased. Because these baits are targeted for cockroaches, no control of ant populations is achieved facilitating increased pest ant problems. The same concept may be responsible for the increase in rank of pest wasps. Pest wasp populations may have benefitted because outdoor perimeter treatments have decreased with IPM adoption.

The fact that head lice were reported as only a medium level pest, ranked between 4.9 and 6.0, should not compel anyone to conclude that they are not a major problem in schools. Many respondents indicated that they ranked head lice low because they felt head lice infestations rarely become the responsibility of pest management staff, falling mostly in the realm of public health and therefore the responsibility of school nurses. Most recent reports indicate that head lice are actually a considerable pest problem in elementary schools (Burgess 1995).

One of the concepts thoroughly emphasized at the workshops was the importance of each district adopting an official pest management policy. Adopting an official policy such as this requires action by the local school board which can become a very cumbersome task if not strongly supported. An increase from 28% to 58% of districts

possessing an official pest management policy is quite remarkable. Possession of an official policy statement is often viewed as the most crucial element of an IPM program (Koehler et al. 1998).

Two additional questions on the survey are very telling. Routine application of pesticides as part of a scheduled program decreased from 76% in 1996 to 36% and 26% in 1997 and 1999, respectively. The percentage of districts reporting using IPM increased from 46% in 1996 to 74% in 1999. This matches well with the number of districts reporting that they do perform routine scheduled spraying of pesticides; 76% in 1996 and only 26% in 1999.

Another important consideration in a School IPM program is who decides where and when pesticides are to be applied. Only individuals properly trained in pest management and pesticide application should be making these types of decisions. According to the 1999 survey, most districts have pest control supervisors (58%) or pest control technicians (62%) making the decision to apply pesticides. However, even in 1999, 30% of districts still have principals making these decisions and 28% of districts have custodians making these decisions. The survey question made no distinction between the application of ready-to-use pesticides and professional-use pesticides and this may have influenced the results. However, in a school environment it is important that only properly trained individuals apply pesticides of any kind, including ready-to-use products. In most schools this would be the School IPM Coordinator or the Pest Management Professional.

Surface sprays were the predominant method of pesticide application in 1996 with 67% of districts responding that surface sprays were being used "most" or "all" of

the time. In 1999 the percentage of districts using surface sprays predominantly dropped to just 21%. This decrease in dependance on surface sprays was paralleled by an increase in bait usage. Only 26% of districts used baits predominantly in 1996 but in 1999, 63% of districts used baits predominantly. This shift in application method was most likely a result of a combination of the School IPM workshops and baiting technology. During 1996-1998 several new bait products were introduced to the pest control industry which have become very popular industry-wide for in-door cockroach control (Miller et al. 1997) replacing surface sprays. Because cockroaches were the top rated pest in schools in 1996 many of the pesticide applications being conducted were probably for this pest.

Frequency of pesticide application changed drastically over the course of the survey period. In 1996, 60% of districts were treating classrooms on some type of scheduled basis. In 1999 this figure decreased to just 24%. Those districts which responded that they do not apply pesticides on a scheduled basis were applying them as-needed (76%). Applying pesticides as- needed (when monitoring mechanisms indicate need and alternatives have been considered) is considered an integral part of a School IPM program. In 1996, 72% of districts responded that they were applying pesticides at least monthly in food service areas. In 1999, only 38% of districts responded they were applying pesticides monthly in food service areas. Food service areas (i.e., cafeterias, dry food storage areas) are considerably better at supporting pest populations and therefore require increased attention by pest management staff and sometimes increased pesticide applications relative to other areas of the school campus.

Perhaps the most surprising response from the surveys was the source of pest management information among respondents. For all three survey years roughly half of

all districts responded that they rely on the CES, pest control contractors, and manufacturers equally for information on pest management. In addition, almost every district responded that they were interested in additional information on IPM each year.

Conclusion. The concept of IPM in urban environments has only begun to be seriously addressed within the last 10 years (Robinson 1996). More specifically, concern over pesticide exposure to children has forced several states to address the issue through increased legislation and regulations (Maryland, Texas, Minnesota., Massachusetts., Louisiana). Some states, such as Indiana, Georgia, and Florida have attempted to rely on IPM becoming implemented on a voluntary basis (Kagans and Feldman 2000). Regardless of implementation mechanism, increased training and education will be critical. Not only will the training for pest management professionals need to be improved but increased education of the school faculty, staff, and administrators will be necessary. Many of the concepts provided within an IPM program are totally new to school staff (Dr. Bobby Corrigan, personal communication). Any significant change will require time and increased training resources.

Two components of an IPM program are crucial for implementation, whether focused on one school, a whole district, or at the state level; training materials and the human resources to provide continued support and encouragement of adoption. Currently, very good materials exist to provide an IPM coordinator the tools necessary to conduct training (Olkowski et al 1991, EPA 1993, Merchant 1996, Koehler et al. 1998) and new materials will certainly continue to be produced. The most important component is the human element. Without dedicated professional positions filled with motivated individuals, IPM implementation will not be successful.

Undoubtedly one of the biggest factors influencing the success of the Florida program has been the creation of the FSIPMAB and the continued participation of its members. Having a Board with every possible concerned party represented where a free exchange of ideas occurs has been challenging but extremely productive. Particularly vital has been the enthusiasm of the Florida Department of Education. In most other states where School IPM has been addressed, the state department of education has not been as much of a key player.

Table 6.1. Summary of program evaluations from participants (n = 505) attending one of five School IPM workshops conducted in 1997.

Evaluation Criteria	Mean[a] ± SD
Location of workshop	4.48 ± 0.55
Classroom comfort	4.36 ± 0.62
Workshop format	4.71 ± 0.46
Workshop content	4.78 ± 0.42
Adequate time allowed	4.50 ± 0.63
Practical value	4.60 ± 0.55
Relevancy	4.63 ± 0.49
Helpfulness of materials	4.76 ± 0.43
Helpfulness of handouts	4.76 ± 0.49
Audio visual materials	4.46 ± 0.67
Instructor's knowledge	4.70 ± 0.40
Instructor's presentation	4.65 ± 0.53
Overall	4.69 ± 0.47

[a]On a scale of 1 - 5 with 1 = least satisfied and 5 = most satisfied.

Table 6-2. Summary of evaluations from 18 County Extension Service Agents attending an In-Service training on School IPM.

Evaluation Question	Response of Participants	
Overall satisfaction with format of workshop.	Very satisfied	83%
	Satisfied	17%
	Undecided	0%
	Dissatisfied	0%
	Very dissatisfied	0%
Would you recommend this workshop to a colleague?	Yes	100%
	No	0%
	Don't know	0%
How knowledgeable were you about the topics covered BEFORE this workshop?	Very knowledgeable	6%
	Moderately knowledgeable	28%
	Fairly knowledgeable	50%
	Slightly knowledgeable	16%
	Not at all knowledgeable	0%
How knowledgeable are you about these topics NOW?	Very knowledgeable	44%
	Moderately knowledgeable	56%
	Fairly knowledgeable	0%
	Slightly knowledgeable	0%
	Not at all knowledgeable	0%

Table 6-3. Response of school district employees in Florida responsible for pest management to 3 surveys[a].

Survey Question	Possible Answers	Response of districts 1996	1997	1999
Does your school district have an official pest management policy statement?	Yes	28%	50%	58%
	No	52%	40%	34%
	Don't know	20%	10%	8%
Margin of error		6%	6%	6%
How does your school district perform pest control **Indoors**?	In-house staff	28%	26%	20%
	Contractor	48%	50%	56%
	Both	24%	24%	24%
Margin of error		6%	6%	6%
How does your school district perform pest control **Outdoors**?	In-house staff	50%	42%	38%
	Contractor	16%	30%	34%
	Both	34%	28%	28%
Margin of error		6%	6%	6%
Does your school district currently use Integrated Pest Management to control pests?	Yes	46%	72%	74%
	No	48%	26%	20%
	Don't know	6%	2%	6%
Margin of error		6%	6%	6%
Does your school district do routine scheduled spraying of pesticides?	Yes	76%	36%	26%
	No	24%	64%	70%
	Don't know	0%	0%	4%
Margin of error		6%	6%	6%
Who makes the decision when and where pesticides will be applied?	Principal	34%	40%	30%
	Designated teacher	0%	2%	0%
	Pest control supervisor	26%	42%	58%
	Pest control technician	42%	50%	62%
	IPM coordinator	16%	36%	34%
	Custodian	32%	32%	28%
	Other	12%	12%	4%
Margin of error		6%	6%	6%

Table 6-3--continued.

Survey Question	Possible Answers	Response of districts 1996	1997	1999
What materials and methods are used for pest management in your district?	Surface/space sprays	74%	66%	52%
	Crack and crevice	72%	86%	90%
	Dust or bait	60%	88%	98%
	Foggers/thermal fog	30%	20%	12%
	Vacuum	12%	32%	28%
	Rodenticides indoors	38%	34%	30%
	Rodenticides outdoors	20%	36%	32%
	Capture devices	44%	74%	78%
Margin of error		6%	6%	6%
What is the frequency of pesticide application in food service areas?	Twice per month	12%	0%	0%
	Monthly	60%	46%	38%
	Quarterly	0%	2%	0%
	Semi-annually	0%	0%	0%
	Annually	0%	0%	0%
	As-needed	28%	52%	62%
Margin of error		6%	6%	6%
What is the frequency of pesticide application in classrooms?	Twice per month	0%	0%	0%
	Monthly	40%	12%	8%
	Quarterly	14%	10%	2%
	Semi-annually	4%	4%	8%
	Annually	2%	6%	6%
	As-needed	40%	67%	76%
Margin of error		6%	7%	6%
What is the frequency of pesticide application in administrative offices?	Twice per month	0%	0%	0%
	Monthly	41%	14%	12%
	Quarterly	18%	10%	15%
	Semi-annually	2%	8%	2%
	Annually	4%	4%	10%
	As-needed	35%	63%	61%
Margin of error		7%	7%	8%

Table 6-3--continued.

Survey Question	Possible Answers	Response of districts 1996	1997	1999
What is the frequency of pesticide application on grounds?	Twice per month	0%	0%	0%
	Monthly	11%	8%	14%
	Quarterly	4%	2%	9%
	Semi-annually	2%	10%	4%
	Annually	2%	0%	0%
	As needed	81%	79%	73%
Margin of error		8%	8%	8%
Who applies pesticides in your district?	Teachers	0%	0%	0%
	Vo-Ag. Teachers	8%	2%	2%
	Custodians	12%	18%	10%
	Maintenance personnel	20%	16%	22%
	In-house pest control staff	30%	32%	34%
	Contracted pest control staff	36%	52%	60%
	School volunteers	0%	0%	0%
	Other	0%	0%	4%
Margin of error		6%	6%	6%
Estimate what extent **Surface Sprays** are used as part of total pesticide treatments in your pest control program.	Not used	11%	19%	18%
	Some of the time	22%	48%	61%
	Most of the time	40%	24%	15%
	All of the time	27%	10%	6%
Margin of error		8%	9%	8%
Estimate what extent **Crack and Crevice** is used as part of total pesticide treatments in your pest control program.	Not used	0%	0%	0%
	Some of the time	45%	58%	76%
	Most of the time	28%	29%	11%
	All of the time	28%	13%	13%
Margin of error		10%	8%	8%
Estimate what extent **Dusts** are used as a part of total pesticide treatments in your pest control program.	Not used	21%	8%	13%
	Some of the time	57%	76%	59%
	Most of the time	18%	16%	23%
	All of the time	4%	0%	5%
Margin of error		14%	11%	12%

Table 6-3--continued.

Survey Question	Possible Answers	Response of districts 1996	1997	1999
Estimate what extent **Baits** are used as part of total pesticide treatments in your pest control program.	Not used	2%	2%	0%
	Some of the time	60%	40%	38%
	Most of the time	19%	45%	39%
	All of the time	7%	14%	24%
Margin of error		11%	9%	8%
Estimate what extent **Granules** are used as part of total pesticide treatments in your pest control program.	Not used	6%	10%	2%
	Some of the time	70%	55%	61%
	Most of the time	6%	33%	28%
	All of the time	18%	3%	9%
Margin of error		12%	10%	8%
Estimate what extent **Aerosols and Fogging** are used as part of total pesticide treatments in your pest control program.	Not used	29%	46%	51%
	Some of the time	69%	49%	49%
	Most of the time	0%	5%	0%
	All of the time	3%	0%	0%
Margin of error		12%	11%	14%
How are most pesticides stored in your district?	District-wide facility	51%	50%	41%
	School-site facility	27%	26%	21%
	No storage by school	22%	24%	39%
Margin of error		6%	8%	6%
Where do you obtain information on pest management?	Cooperative Extension	56%	64%	67%
	Pest control contractor	52%	68%	59%
	Vendors/Manufacturers	52%	60%	50%
	Other	18%	12%	27%
Margin of error		6%	6%	6%
Do you want additional information on IPM?	Yes	96%	90%	91%
	No	4%	10%	9%
Margin of error		6%	6%	6%

Margin of error = ± 95% confidence interval
[a]Overall response rate was 75%

Table 6-4. Rank (10 = most important, 1 = least important) of pests as reported in 3 surveys of school districts. Numbers in parentheses following years indicate number of school districts represented out of 50.

	Mean ± SE rank by year of survey		
Pest	1996 (39)	1997 (42)	1999 (45)
Ants	8.9 ± 0.2	9.1 ± 0.2	9.2 ± 0.2
Cockroaches	9.5 ± 0.2	9.2 ± 0.2	8.3 ± 0.3
Head lice	5.6 ± 0.4	6.0 ± 0.3	4.9 ± 0.3
Landscape pests	4.3 ± 0.3	3.9 ± 0.3	3.8 ± 0.3
Rodents	6.3 ± 0.3	6.6 ± 0.3	6.9 ± 0.3
Snakes	3.2 ± 0.3	3.3 ± 0.2	3.0 ± 0.2
Spiders	5.6 ± 0.3	5.5 ± 0.2	5.2 ± 0.3
Wasps	6.1 ± 0.3	6.3 ± 0.2	7.2 ± 0.2
Weeds	4.5 ± 0.3	4.1 ± 0.4	4.9 ± 0.4

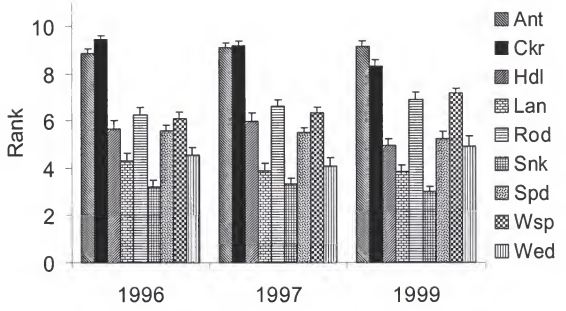

Fig. 6-1. Ranking (10 = most important, 1 = least important) of major pests in schools as reported in 3 surveys of school district pest management personnel, Ant, ants; Ckr, cockroaches; Hdl, head lice; Lan, landscape pests; Rod, rodents; Snk, snakes; Spd, spiders; Wsp, wasps; Wed, weeds.

CHAPTER 7
DOCUMENTATION OF PEST MANAGEMENT IN A SCHOOL AND PROPOSED APPLICATION OF SPATIAL ANALYSIS TO DETERMINE RISK

Introduction

Children are faced with various hazards in the school environment. Two of these potential risks are associated with managing insect pests in the school; pests and pesticides. Pests present the potential dangers of stings, bites, and disease (Goddard 1999), asthma from allergens (Brenner et al. 1993, Rosenstreich et al. 1997), and simple emotional stress generated from fear. To combat pests, pest management staff employ the use of various pest control techniques including the use of pesticides (Mallis 1997). Contact with pesticides, however, may present additional risks through acute exposure (skin or eye irritation) and/or chronic exposure (nervous system disorders) (NRC 1980).

Children have been identified as being at a higher risk to pesticide exposure because of their lower body weight and unique behavior (NRC 1993). In response to growing concern the U.S. Environmental Protection Agency (EPA 1993) has encouraged schools to adopt integrated pest management to help reduce potential risks to children. In addition, many states have mandated IPM be used in all schools state-wide (Owens and Feldman 1998) and federal legislation has been proposed (SEPA 1999).

IPM has been successfully used in agriculture to reduce the reliance on pesticide use for decades (Pedigo 1989). However, little documentation exists describing pesticide use in schools. It is the purpose of this research to fully document qualitative and

quantitative data regarding pesticide use and pest infestations in a typical public school and determine potential uses of spatial analysis technology in describing the in-door risks.

Materials and Methods

Experiment Site. The P.K. Yonge Laboratory school is part of the Alachua County School System in Gainesville, Florida. P.K. Yonge is unusual in that it is a laboratory school associated with the University of Florida College of Education and includes grades kindergarten through 12th grade. The campus contains typical building elements found in most schools including classrooms, a cafeteria, a gymnasium, an auditorium, athletic fields, and an administrative complex.

Historical Data. Information regarding child distribution and the time children spend in various places on campus was obtained through school records and during interviews with members of the faculty and principal's staff. Records of pest sightings and pesticide applications were obtained through the University of Florida Department of Environmental Health and Safety which is responsible for pest management on the P.K. Yonge campus.

Predicted Risk Assessment. The kindergarten classroom used for spatial analysis was 36 ft. x 36 ft. The classroom contained elements found in a typical kindergarten classroom including tables with chairs, a piano, sink, separate boy's and girl's restrooms, and spaces on the floor designated for sitting during story-time.

All spatial images and data isolines (surface maps) were produced using ArcView Geographical Information Systems Software (ver. 3.1, Environmental Systems Research

Institute, Inc., Redlands, CA). ArcView is a data management and spatial analysis software package which can utilize GIS information and spatial statistics to interpret spatial data. Originally designed for geological applications (Isaaks and Srivastava 1989), spatial statistics has become popular with entomologists/ecologists (Rossi et al. 1992) interested in predicting or modeling insect populations (Liebhold et al. 1993) and improving pest management decision-making particularly in urban environments (Brenner et al. 1998a, Focks et al. 1999).

Within the classroom, a rough grid of simulated sampling points was established as the source of estimated data (Fig. 7-3). For each of these sampling points an initial rank of 1 - 5 was generated based on estimated probability of the presence of each factor. A rank of 1 = 0 - 20% probability of the factor being present while a rank of 5 = 81 - 100% probability. All data submitted for analysis is estimated data based on assumptions for each of the three factors.

Estimated pesticide distribution in the kindergarten classroom was based on data collected from pest management records. The total amount of pesticide applied in the classroom was distributed across the spatial grid based on typical "baseboard" treatment expectations (Snell 1997). Complete surface maps are developed by the statistical package (using inverse distance to power as a gridding method) based on the known quantitative data and assumed spatial patterns through interpolation. Child/hour data (a unit describing the presence of a child in space for 1 hour) for the kindergarten classroom was obtained through personal observation and from the specific teacher of that classroom during an interview. The total number of child/hours generated in the kindergarten classroom was 120 per day (20 children x 6 hrs). As in the pesticide data,

the statistical package completes the estimated distribution of child/hours across the entire room based on interpolation. Pest infestation levels were based on the estimated likelihood of pest infestation relative to distances to water sources or food sources located within the classroom. Surface maps representing interactions (grid math) of the above factors were the result of multiplying or subtracting ranks among the factors (Isaaks and Srivastava 1989).

Results

Historical Data. Total enrollment for the P.K. Yonge Laboratory school is approximately 1100 students accumulating 6,946 child/hours per day. The three primary locations generating the most child/hours are the classrooms for grades 6-12 (39.0%), grades K-5 (25.7%), and the cafeteria (8.2%). All other locations combine to generate 27.1 % of the total child/hours. Amount of pesticides applied across the entire campus, expressed in total grams of active ingredient (a.i.), was 899.4, 783.9, and 311.3 for the years 1993, 1995, and 1997 respectively (Table 7-1). These active ingredients were applied as 37 different formulated products including emulsifiable concentrates, wettable powders, granules, baits, and dusts. Non-pesticidal components consisted primarily of monitoring devices, sticky traps, and glue boards. Total a.i. applied as formulated baits or organophosphate/carbamate formulations changed considerably over the 5 year period (Table 7-2). Pest reports (indicating presence of pests) were generated by (in decreasing frequency) cockroaches, ants, bees, wasps, weeds, termites, mosquitoes, birds, mole crickets, rodents and other miscellaneous pests. A comparison of child/hours, pest

reports, and pesticide applications (Fig. 7-1) seems to indicate a linear relationship among these three factors.

Predicted risk assessment. The boundary of P.K. Yonge campus and its buildings appear overlaid on an aerial photograph of the campus in Fig. 7-2. The kindergarten classroom (H123) that served as our base experiment with georeferenced floorplans and all 135 simulated sampling locations appears in Fig. 7-3.

For all surface maps, darker contour lines represent higher probability of the appropriate factor being present (higher rank) and lighter contour lines represent lower probability (lower rank) of the appropriate factor being present. Fig. 7-4 represents the estimated spatial distribution of child/hours generated in one day in the experimental kindergarten classroom. Four locations generate the majority of child/hours; the drawing tables, the two story-time locations, and the entrance to the room. Fig. 7-5 represents the estimated spatial distribution of likely pest infestation based on distance to a water source. Pests would be primarily distributed in four locations; the corner of the room next to the rear entrance and the trash receptacle, the front entrance, and the two bathrooms. Fig. 7-6 represents the predicted potential spatial interaction of pests and children (pest risk) in the classroom. There are primarily two locations where the pest risk is generated; at the room entrance and the area near the drawing tables. Fig. 7-7 represents predicted pesticide distribution within the room following a "baseboard" treatment. The pesticide is distributed all around the room but primarily along the surface of the room where the wall and floor meet. Fig. 7-8 represents the spatial interaction of predicted pesticide and child/hours distribution (pesticide risk). Pesticide risk is generated primarily in four locations; the entrance, the two story-time areas, and

near the drawing tables. Addition of both risk estimates provides an assessment of overall total risk being generated within this kindergarten classroom (Fig. 7-9). Total risk is primarily in three locations; the drawing table area, the story-time area, and the front entrance. Fig. 7-10 represents the interaction of predicted pesticide and pest distribution (treatment benefit). Treatment benefit occurs primarily at two locations; the front entrance and the area next to the trash receptacle and rear entrance. Ranks of benefit subtracted from pest risk provide a contour (Fig. 7-11) describing the comparison of benefit to risk.

Discussion

Historical data. The historical data clearly indicate a change in pesticide use in the amount of total a.i. applied and in the type of formulation employed. Relative levels of child/hours, pest reports, and pesticide applications are equally distributed across the three areas examined; K-5, 6-12, and the cafeteria. This suggests that there is a linear relationship between child/hours, pest reports, and pesticide applications. Wherever children are is where the pest reports are generated which results in the location being treated. With no additional information, it might be concluded that no possible solution exists to reduce the potential hazard of pest/pesticide exposure to the children in the school. However, looking more closely at the specific interactions between the three factors in space and time using simple spatial statistics suggests that precision targeting treatments could greatly reduce the risks.

Spatial data. The greatest resolution obtainable with historical records indicates that children, pests, and pesticides occur in the same locations. However, the increased

resolution provided by spatial analysis allows us to determine that some separation exists within a classroom between these three factors (Figs. 7-6 and 7-8). There are specific locations where pests are present but no children. However, traditional pest control methods involving space sprays and baseboard treatments do not allow the pests to be targeted exclusive of the children (Fig. 7-8).

Traditional pest control methods in the current model did not completely address pest infestation (Fig. 7-11). This would suggest that a monitoring program to aid in identifying specific locations where pests are present could greatly improve targeting pesticide applications (Brenner et al. 1998). In addition, the use of pesticides formulated as baits would greatly reduce overall pesticide distribution and therefore reduce potential pesticide risk to children.

The model described here is a simple application of spatial analysis to aid in visualization of risks generated in a classroom. Limits to this methodology exist. More rigorous studies are needed to accurately identify and characterize covariates predictive for likelihood of pest infestation and pesticide distribution. Additional features not addressed in the current study include influence of pests located outdoors. If pests can be monitored outdoors and subsequently treated, this would lower the level of pest infestation indoors. In the current study, all children are assessed equally. In reality there may be a mechanism to qualitatively define "at-risk" children who possess allergies, suppressed immune systems, or chemical sensitivities and input this as part of the overall risk assessment.

In order to properly quantify risks using a spatial analysis tool, surface maps with contours would need to be replaced with maps based on probability values within each

cell of the interpolated grid. This adaption would allow development of specific algorithms to quantify spatial risks. Application of these tools would help schools to efficiently allocate resources designated for pest management and improve overall environmental health.

There are three components contributing to the risks and benefits of pest management in schools (children, pests, and pesticides). These three components make up the corners of the IPM Risk Management Triangle with each interaction creating a leg of the triangle. The interaction of children and pests generates a pest-child risk factor. The interaction of children and pesticides generates a pesticide-child risk factor. The interaction of pesticides and pests generates a pesticide-pest benefit factor.

Unfortunately, only one of the factors present in the IPM Risk Management triangle (pesticide-child risk) is currently attracting attention from legislators (SEPA 1999). Although the pesticide-child risk factor currently generates the most concern, the ultimate solution to pest management in schools will adequately address all three factors of the IPM Risk Management Triangle.

Some hesitation remains among the scientific community (Robinson 1996) regarding the application of IPM into urban environments. However, the results provided in this study indicate the great potential IPM may hold in reducing risks in school environments.

Table 7-1. Number of pest reports (% of total) for P.K. Yonge School by pest.

Pest	Number of complaints by year (%) 1993	1995	1997
Ants	27 (45.0)	18 (34.6)	25 (46.3)
Cockroaches	19 (31.7)	11 (21.2)	3 (5.6)
Rodents	3 (5.0)	12 (23.1)	2 (3.7)
Other vertebrates	1 (1.7)	1 (1.9)	3 (5.6)
Wasps and Bees	10 (16.7)	10 (19.2)	18 (33.3)
Other	-	-	3 (5.6)
Total	60 (100)	52 (100)	54 (100)

Table 7-2. Quantity of pesticides applied to P.K. Yonge School.

Type of Pesticide	Grams of A.I. applied by year		
	1993	1995	1997
Organophosphate/Carbamate[a] (% change)	809.2	681.6 (-15.8)	286.9 (-60.0)
Pyrethrins/pyrethroids[b] (% change)	25.9	16.7 (-35.5)	12.3 (-26.3)
Baits[c] (% change)	2.9	6.4 (+120.1)	12.2 (+90.6)
Other[d] (% change)	61.4	79.2 (+29.0)	0.0 (-100.0)
Total (% change)	899.4	783.9 (-12.8)	311.3 (-60.2)

[a]Includes all products which contain organophosphates or carbamates as active ingredients
[b]Includes all products which contain pyrethrins or pyrethroids as active ingredients
[c]Includes hydramethylnon, sulfluramid, and borates as bait formulations only
[d]Inlcudes insect growth regulators, inorganics, and botanicals

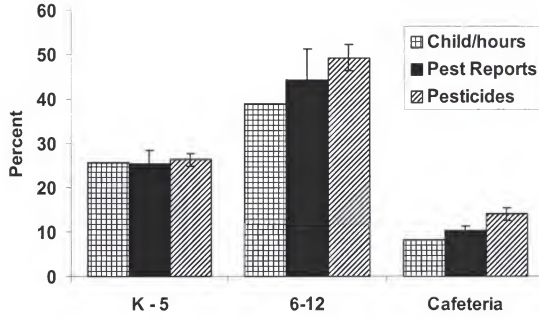

Figure 7-1. Location of time spent in classrooms by children, pest problem reports, and pesticide applications at P.K. Yonge campus averaged for 1993, 1995, and 1997.

Fig. 7-2. An aerial photograph of P.K. Yonge School in Gainesville, Florida. The green line represents the border of the campus and the buildings are outlined in brown.

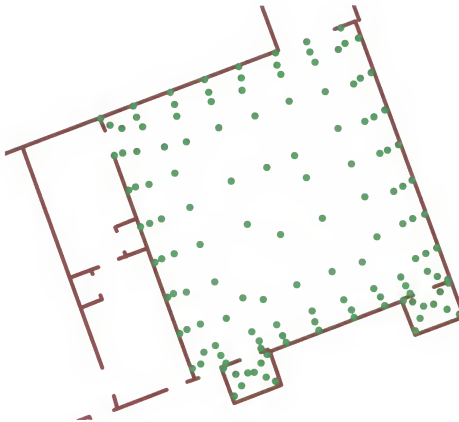

Fig. 7-3. The experimental kindergarten classroom (H123) on P.K. Yonge campus. The green dots represent simulated sampling points used to estimate levels of various parameters.

Fig. 7-4. Predicted distribution of child/hours (as ranks) within the classroom. The darkest contours represent areas of the classroom where children spend the most time.

Pkfloorplandd.shp
Distribution of Child Hours
1
1.01 - 1.99
2 - 2.99
3 - 3.99
4 - 5

Fig. 7-5. Predicted pest distribution (as ranks) within the classroom based on location of food and water sources.

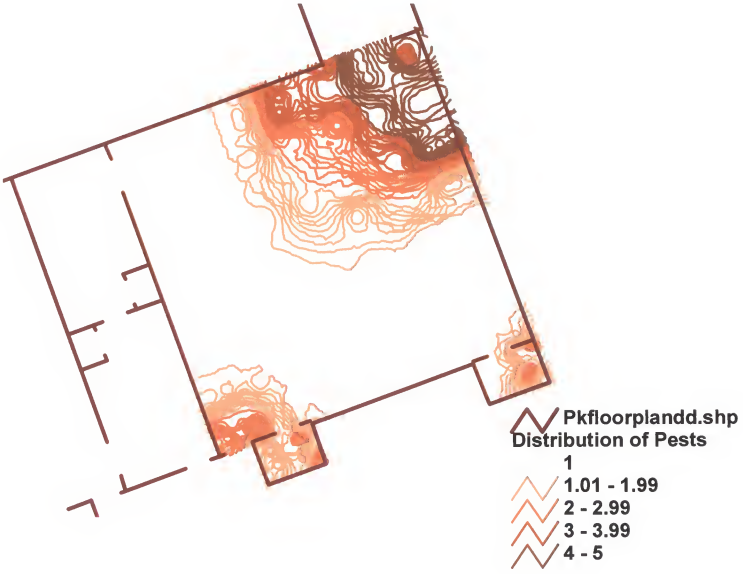
Pkfloorplandd.shp
Distribution of Pests
1
1.01 - 1.99
2 - 2.99
3 - 3.99
4 - 5

Fig. 7-6. Predicted distribution of pest risk (as a ranking) within the classroom. Contours represent the interaction of pests and children.

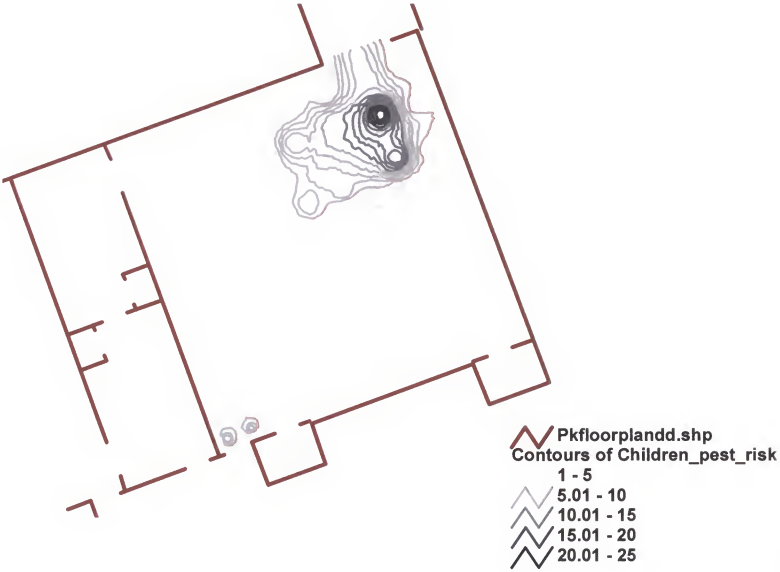
Pkfloorplandd.shp
Contours of Children_pest_risk
1 - 5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25

Fig. 7-7. Predicted pesticide distribution (as a ranking) based on a typical baseboard application.

Pkfloorplandd.shp
Distribution of Pesticide
1
1.01 - 1.99
2 - 2.99
3 - 3.99
4 - 5

Fig. 7-8. Predicted distribution of pesticide exposure risk (as a ranking).

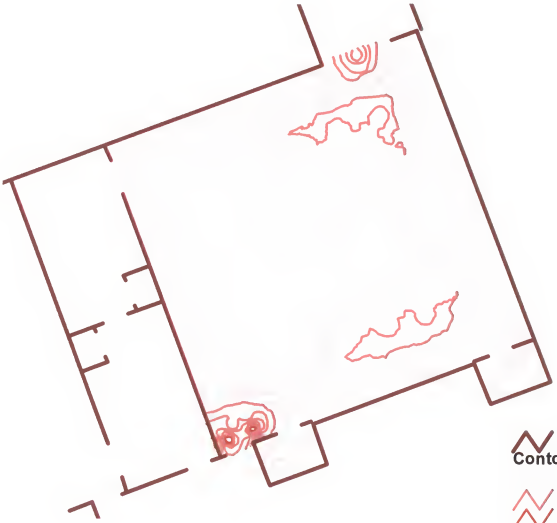
Pkfloorplandd.shp
Contours of Pesticide_child_risk
1-5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25

Fig. 7-9. Predicted distribution of total risk (as a ranking). Total risk is the sum of pest exposure risk and pesticide exposure risk.

Pkfloorplandd.shp
Contours of Total Risk
1 - 5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25

Fig. 7-10. Predicted distribution of benefit resulting from the interaction of estimated pesticide distribution and pest distribution (as a ranking) within the classroom.

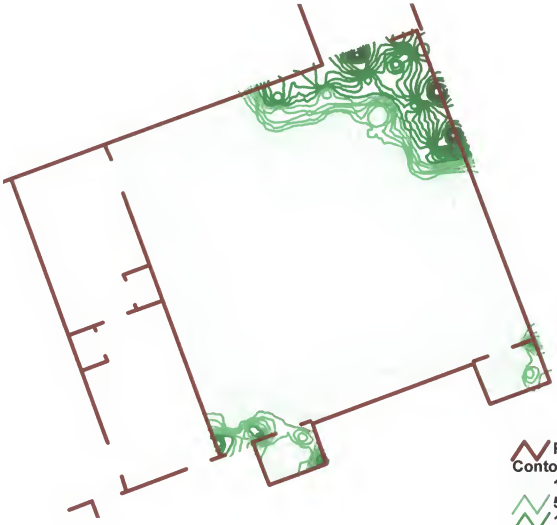
Pkfloorplandd.shp
Contours of pesticide_pest_benefit
1 - 5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25

Fig. 7-11. Predicted distribution of benefit compared to predicted distribution of total risk (as a ranking) within the classroom.

Pkfloorplandd.shp
Contours of Comparison Benefit : Total Risk
-20 - -15.01
-15 - -10.01
-10 - -5.01
-5 - -0.01
0.01 - 5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25

CHAPTER 8
SUMMARY

Children spend 1/6th of their adolescent life on school campuses. Within the school environment children are exposed to a wide variety of health hazards. A significant risk facing children in schools is associated with pests. Both insect pests and vertebrate pests present children with the potential threat of bites, stings, and disease. Recent research reports have concluded that allergens produced by cockroaches are the single most important causal agent for asthma in children.

In response to the potential threat of pest-generated risks schools rely on pest management staff to address pest infestations. Many pest management professionals utilize pesticides to aid in pest suppression. Pesticides applied in-doors reduce levels of cockroaches, ants, fleas, and rodents. Out-doors pesticides are applied to combat ants, turf pests, ornamental plant pests, and vertebrate pests.

Pesticides, however introduce additional risk into the school system. When contacted by students, staff, or faculty some pesticides can cause acute or chronic health problems. A concept developed to address both types of risk, Integrated Pest Management (IPM), has been introduced to the Florida school system to improve the school environment for children.

A series of IPM training workshops directed towards school employees and the commercial pest control industry was conducted during 1997 at five locations around

Florida. Workshop topics included the overall concept of IPM (to reduce pest and pesticide generated risks), specific control strategies of important pests for long term suppression, pest prevention techniques, reduced-risk treatment methods, and the importance of communication in an IPM program. Over six hundred people attended the workshops and the feedback was positive.

As a result of the workshops, 58% of Florida school districts have adopted pest management policy statements, only 26% of school districts continue to apply pesticides on a calendar basis, and 74% of school districts have adopted IPM. In addition, a World Wide Web Site was established to deliver educational materials to interested parties. The National School IPM WWW Site is available at http://www.ifas.ufl.edu/~schoolipm/ This web site has become the leading source for delivering School IPM information across the United States.

As an example of the change in trends occurring in Florida schools, P.K. Yonge School in Gainesville, FL was selected for an experiment. Examination of historical pest management records revealed that overall use of pesticides dropped 65% from 1995 to 1997 as IPM was adopted into the school. Additionally, most indoor applications of pesticides were in the form of baits by 1997. A closer inspection of child activity, pest distribution, and pesticide distribution within a single kindergarten classroom using spatial analysis validated the risk reduction potential of monitoring and precision targeting pests. Within the confines of a typical classroom, it is possible to manage pests at adequate levels while not introducing additional pesticide exposure hazards.

An important insect pest most prevalent among school children is the human head louse, *Pediculus capitis* De Geer. In Alachua County schools, chronic cases of

pediculosis are predominant among girls in the 4th grade. Assuming that children chronically infested with head lice tend to serve as the main source of infestation for additional children, school nurses could improve management programs by targeting such children during screening programs.

Treatment for head lice often involves application of an insecticidal shampoo. The most common brands of pediculicides containing pyrethroids or synergized pryrethrins currently exhibit much lower efficacy than when initially marketed. Recent research reported from other sources in the U.S. and Europe confirm the presence of resistance to pyrethroids in head lice. This should give rise to expedited research into treatment alternatives as most over-the-counter pediculicides contain pyrethroids (or pyrethrins) as active ingredients. Fipronil and imidacloprid, exhibiting relatively low mammalian toxicity, present interesting candidates for future head lice toxicants.

Many parents, teachers, and school officials become concerned about head lice being transmitted from carpet, desks, and other objects present in the school. In order to help determine the survival ability of the head louse, once removed from the host, studies were conducted exposing recently fed head lice to three different temperatures, 20, 25, and 30°C. Head lice died very quickly at 30°C having an LD_{50} of about 14 hrs. At 20 and 25°C the LD_{50} was 25 and 21°C respectively. These results suggest that at room temperatures (20 and 25°C) recently fed lice can survive overnight on the carpet of a school but not over the period of a weekend. However, head lice eggs hatched up to 9 days after being removed from the host and exposed to both 25 and 30°C. No eggs hatched at 20°C. To reduce potential of dislodged lice infesting an uninfested child,

schools should integrate vacuuming as part of a standard procedure in classrooms where infested children have been identified.

The human head louse and the human body louse, *P. humanus* L., are nearly indistinguishable and are still considered subspecies by some researchers. Analysis of cuticular hydrocarbons revealed that a completely different pattern exists with each species. Specifically the four hydrocarbons with retention indexes of 2500, 2700, 2900, and 3075 can be used to distinguish between the two species of *Pediculus*.

Using various microscopic methods several morphological characters of the head louse were examined and documented. The translucent nature of the cuticle of the head louse make it especially appealing to observe using a dissecting or compound microscope. Tracheal tubes and developing eggs within a female louse are clearly visible through the cuticle of the insect. In addition, the gonopods, spiracles, details of the antennae, mouthparts, and external genetalia of the male louse are visible using a scanning electron microscope.

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BIOGRAPHICAL SKETCH

Clay W. Scherer was born March 12, 1971, in West Palm Beach, Florida, to parents Anne and Les Scherer. Clay grew up in Stuart, Florida, and graduated from Martin County High School. In the fall of 1989 Clay enrolled at the University of Central Florida to become an engineer. It was not long before he changed his mind and his major and ultimately graduated with a B.S. in biology in 1994. From there, Clay was provided the opportunity to attend graduate school at the University of Florida in entomology under the tutelage of Dr. John Capinera. During this time Clay met Dr. Phil Koehler and became interested in the potential of pursuing a Ph.D. in Dr. Koehler's program upon finishing his M.S. In May of 1997 Clay received his M.S. degree and subsequently joined Dr. Koehler's Urban Entomology Laboratory as a Ph.D. student.

At about the same time the Florida Department of Education approached Dr. Koehler about starting a program to improve pest management in Florida schools. These initial discussions resulted in Dr. Koehler assigning Clay to work on the School Integrated Pest Management project as part of his Ph.D. program. Upon graduation Clay intends on pursuing a career in the chemical manufacturing industry and sharing the rest of his life with his fiancée, Ms. Juliann Hickey.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Philip G. Koehler, Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard J. Brenner
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John L. Capinera
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard J. Lutz
Professor of Marketing

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2001

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